TRACE ELEMENT ACCUMULATION IN LOTIC SYSTEMS: IMPLICATIONS FOR AQUATIC ORGANISMS AND HUMAN HEALTH

by

GRETCHEN LOEFFLER PELTIER

(Under the direction of Judith L. Meyer)

ABSTRACT

Current understanding of trace element accumulation in lotic food webs is lacking in comparison to the breadth of research in lentic systems. However, as urbanization increases, rivers receive greater loading of trace elements associated with point and nonpoint sources of contamination. Using C*orbicula fluminea,* I related trace element concentrations (nickel, copper, zinc, arsenic, selenium, cadmium, mercury, and lead) in clam tissue to particular point and nonpoint sources in an urban river network. Concentrations of arsenic and selenium were highest in clams from sites downstream of coal-fired power plants (CFPPs). Mercury and cadmium concentrations were higher in clams from sites with forested and urban catchments, respectively. I further explored the accumulation patterns of trace elements in clams by transplanting clams from a reference stream into a stream contaminated with CFPP discharge. Clams accumulated significantly higher concentrations of most trace elements (e.g, arsenic, selenium), higher growth rates, and elevated glutathione concentrations at the most contaminated site. Mercury concentrations declined in clams that were transplanted into the selenium-rich environment at the most contaminated site. To determine if trace element accumulation patterns reflect widespread food web contamination, I sampled clams and representative components of the food web (basal resources, invertebrates, and fishes) in two streams, one contaminated with CFPP discharge and one reference. Clams and all food web components in the contaminated stream had significantly higher concentrations of trace elements except mercury, whose concentration in clams and the rest of the food web in the reference stream were consistently higher than in the contaminated stream.

Aquatic food webs and humans are linked through the pathway of fish consumption. If food webs are contaminated with trace elements, anglers may be exposed to concentrations that exceed human health standards. By measuring trace element concentrations in *Lepomis macrochirus* at four sites in an urban river and relating those to fish consumption patterns by anglers, I assessed the relative hazard to human health. Arsenic concentrations in muscle fillets exceed current fish consumption guidelines. The results of this dissertation support implementation of tissue-based criteria for trace elements in wildlife and subsequent review of current TMDL assessments for rivers.

INDEX WORDS: Urban, Coal ash, Selenium, Arsenic, Mercury, Cadmium, Nickel, Zinc, Lead, Copper, *Corbicula fluminea*, *Lepomis macrochirus*, Food web, Human health

TRACE ELEMENT ACCUMULATION IN LOTIC SYSTEMS: IMPLICATIONS FOR AQUATIC ORGANISMS AND HUMAN HEALTH

by

GRETCHEN LOEFFLER PELTIER

B.S., Washington University, 1997

M.P.H., Columbia University, 2001

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2006

TRACE ELEMENT ACCUMULATION IN LOTIC SYSTEMS: IMPLICATIONS FOR AQUATIC ORGANISMS AND HUMAN HEALTH

By

GRETCHEN LOEFFLER PELTIER

Major Professor: Judith L. Meyer

Committeee: Laurie A. Fowler
William A. Hopkins
Charles H. Jagoe
Randall O. Manning

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2006

ACKNOWLEDGEMENTS

Numerous people have contributed to the completion of this dissertation and I am truly thankful for their guidance and expertise. The faculty, staff, and students of the Institute of Ecology at the University of Georgia have been incredibly supportive during my academic career. The Meyer, Rosemond, Freeman, and Hopkins lab groups deserve many thanks for providing lab space, supplies, field assistance, and advice over the years. Past and present Meyerfauna folks provided me with critical feedback and support at each step of the dissertation process plus they really know how to make me laugh! Deanna Conners taught me how to find *Corbicula* in the field, keep them caged in streams, and the intricacies of glutathione analysis. She has been an incredible resource and sounding board for ideas.

Special thanks to my committee (Judy Meyer, Bill Hopkins, Randy Manning, Laurie Fowler, and Chuck Jagoe) for their time, guidance, and encouragement throughout my studies. I feel fortunate to have had the opportunity to work with Judy. She has taught me a great deal about ecology and encouraged my desire to integrate the fields of public health and ecology in my research. Bill has also been instrumental to my academic success. He has provided me with financial support and lab facilities at the Savannah River Ecology Laboratory as well as constructive feedback on many aspects of this dissertation from start to finish.

I would like to acknowledge Birgit Bolton, Sally Bethea, Harlan Trammell, and Skelly Holmbeck-Pelham of the Upper Chattahoochee Riverkeeper. They provided financial support during part of my career, access to the river, and the opportunity for me to use my public health background for real health concerns in the Chattahoochee River.

Many thanks to my family for their support during my career, and I know that our weekend outings to the mountains and local streams during my childhood instilled a love for the environment at an early age. I also owe an incredible debt of gratitude to my husband and best friend, Rick. He has trekked through urban streams, analyzed samples, and been willing to help with whatever else I needed. I want to thank him for his patience, encouragement, and support which have kept me going throughout this process. Most of all, I want to thank him for his smile and laughter which brighten my life.

TABLE OF CONTENTS

PAGE

CHAPTER 1

INTRODUCTION

Human populations are increasing in urban areas, and the extent of urban regions continues to expand. In 1800, only 2% of the population lived in urban areas, whereas in 2000, nearly 50% of the world's population lived in cities (Cohen 2003). Increased urbanization is associated with chemical, hydrological, and physical changes in rivers and streams. The term urban stream syndrome describes the patterns of chemical, physical, and biological degradation associated with increased urbanization in a catchment (Meyer et al. 2005). One component of the urban stream syndrome is elevated concentrations of metals (e.g. zinc, copper, cadmium) and metalloids (e.g. arsenic and selenium) which I term "trace elements" in this dissertation. These arise from various point and nonpoint sources including wastewater treatment plants, stormwater runoff, and industrial activities (Lenat and Crawford 1994, Horowitz et al. 1999, Paul and Meyer 2001).

One point source, coal-fired power plant (CFPP) discharge, is a significant contributor to trace element inputs in lentic and lotic environments. CFPPs supply half of the electricity required to meet the energy demands of the United States (Rowe et al. 2002). The process of generating electricity from coal combustion involves stages of volatilization and transformation of coal, precipitation of the bottom and fly ash, and formation of aerosols. Coal combustion waste contains trace elements such as nickel, copper, zinc, arsenic, selenium, cadmium, mercury, and lead, and it is disposed into landfills, aquatic basins, and minefills (Rowe et al. 2002). In 1999, EPA estimated that there were approximately 600 landfills and surface impoundments in operation at 450 CFPPs across the country (NRC 2006). Surface impoundments or aquatic basins receive coal combustion wastes sluiced with water. The solid or particulate fraction settles to the bottom of the impoundment and the resulting bottom slurry may be dewatered and transported to a landfill as the impoundment fills with solids (NRC 2006). Discharges from the clear surface waters of the impoundment typically empty into neighboring rivers or streams to maintain pond level.

These contaminants have the potential to be transferred through the food web in streams and rivers with elevated trace element concentrations. Lake chubsuckers (*Erimyzon sucetta*) exposed to coal ash sediments had a high incidence of mortality and severe fin erosion (Hopkins et al. 2000). In the Coeur d'Alene River Basin, an area impacted by mining waste, macroinvertebrates represented a concentrated source of metals that was transferred through trophic levels leading to lethal diets in fish populations (Farag et al. 1998). Reductions in invertebrate community biomass, species diversity, and metal-sensitive species occurred downstream of inputs from a western mine (Beltman et al. 1999). The majority of knowledge about trace elements in aquatic systems has been acquired through the study of mining activities (Clements 1994, Farag et al. 1998, Beltman et al. 1999, Clements et al. 2000, Mason et al. 2000, Hamilton and Buhl 2004) and lentic systems receiving coal ash effluent (Lemly 1997, Hopkins et al. 2000, Lohner et al. 2001a, Lohner et al. 2001b, Rowe et al. 2001, Jackson et al. 2002, Lemly 2002, Rowe et al. 2002, Hopkins et al. 2004b).

Three CFPPs discharge into the section of Chattahoochee River that flows through metropolitan Atlanta, Georgia. Elevated dissolved and particulate concentrations of arsenic and selenium were found in the Chattahoochee River, but these concentrations decrease approximately 20 km downstream of two CFPP effluent discharges (Froelich and Lesley 2001, Lesley and Froelich 2003). Previous research has shown elevated concentrations of trace elements in macroinvertebrates and fishes throughout the Chattahoochee River, but a relationship between potential sources and elevated concentrations has not been clearly established (Rosi-Marshall 2002). Over half of fish collected from the Chattahoochee River had total mercury concentrations in their muscle fillets triggering EPA recommendations of no more than three 8oz meals per month for adult males and non-pregnant females (Rosi-Marshall 2002). In 2005, Georgia EPD issued recommendations to limit consumption of several fishes in the Chattahoochee River including largemouth bass, carp, and bluegill because of mercury and PCB contamination. However, during routine river patrols, Upper Chattahoochee Riverkeeper (UCR) staff have noted a high frequency of anglers fishing along the Chattahoochee River and keeping their catch for later consumption (Loeffler et al. 2003).

In rivers, contaminant inputs are diluted and transported to downstream reaches. It is thought that lotic systems can tolerate greater loading of contaminants than lentic systems (Simmons and Wallschlager 2005, Orr et al. 2006). However, current trace element research primarily focuses on processes in lentic systems (Rowe et al. 2002, Simmons and Wallschlager 2005). Further understanding of trace element accumulation in lotic food webs receiving multiple sources of contamination is needed (e.g., urban streams receiving stormwater runoff as well as wastewater and coal-fired power plant discharges). My dissertation research, which focuses on trace element inputs in urban rivers, will contribute to the understanding of trace element accumulation patterns in lotic systems. If the patterns associated with loading are understood, then we will be able to more adequately protect aquatic organisms and public health. In this dissertation, I address trace element accumulation in an indicator organism (*Corbicula fluminea*), *Lepomis macrochirus,* and two lotic food webs. I address four specific questions:

- Are trace element concentrations in tissues of an indicator organism (*Corbicula fluminea*) related to point and nonpoint sources of trace elements in a river network (Chapter 2)?
- What are the temporal patterns of trace element accumulation and their consequences for growth in *Corbicula fluminea* living in a stream receiving discharge from a coal-fired power plant (Chapter 3)?
- Are trace element concentrations in *Corbicula fluminea* indicative of more widespread trace element contamination in a stream food web (Chapter 4)?
- Do muscle concentrations of trace elements differ from concentrations in liver tissue in bluegill caught at several sites along an urban river? Do the concentrations of trace elements constitute a public health hazard to anglers (Chapter 5)?

Further study of the behavior of trace elements in lotic environments is crucial for the protection of both ecosystem and human health. Considerable scientific research has assessed the risk to human health from exposure to trace elements through air and water. Based on this research, regulatory guidelines have been established for maximum contaminant levels in water and limits on air emissions from combustion processes. Despite the strength of the human health assessments, establishment of water, tissue, or taxon-specific criteria for contaminants continues. Since 1985, EPA guidelines for aquatic life criteria have relied on toxicity data and exposures through water which do not account for dietary exposure. Development of tissue-based criteria for metals in aquatic systems rather than criteria based solely on dissolved concentrations will more accurately predict the effects of these contaminants on aquatic populations and communities. Greater understanding of ecological pathways resulting in accumulation of contaminants in aquatic organisms will provide a strong argument in the regulatory decision-making process and result in more integrative approaches to ecosystem protection. This dissertation will quantify accumulation patterns in lotic systems receiving trace element loads, and a better understanding of these patterns will facilitate improved protection of aquatic organisms and public health.

Literature Cited

Beltman, D. J., W. H. Clements, J. Lipton, and D. Cacela. 1999. Benthic invertebrate metals exposure, accumulation, and community-level effects downstream from a hard-rock mine site. Environmental Toxicology and Chemistry **18**:299-307.

Clements, W. H. 1994. Benthic invertebrate community responses to heavy-metals in the Upper Arkansas River basin, Colorado. Journal of the North American Benthological Society **13**:30-44.

Clements, W. H., D. M. Carlisle, J. M. Lazorchak, and P. C. Johnson. 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecological Applications **10**:626-638.

Cohen, J. E. 2003. Human population: The next half century. Science **302**:1172-1175.

Farag, A. M., D. F. Woodward, J. N. Goldstein, W. Brumbaugh, and J. S. Meyer. 1998. Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River Basin, Idaho. Archives of Environmental Contamination and Toxicology **34**:119-127.

Froelich, P. N., and P. Lesley. 2001. Tracing germanium contamination from coal-fired power plants along the Chattahoochee River-Apalachicola River: implications for the toxic metalloids arsenic and selenium. *in* K. J. Hatcher, editor. Proceedings of the Georgia Water Resources Conference. Institute of Ecology, University of Georgia, Athens, GA.

Hamilton, S. J., and K. J. Buhl. 2004. Selenium in water, sediment, plants, invertebrates, and fish in the blackfoot river drainage. Water Air and Soil Pollution **159**:2-34.

Hopkins, W. A., J. W. Snodgrass, J. H. Roe, B. P. Jackson, J. C. Gariboldi, and J. D. Congdon. 2000. Detrimental effects associated with trace element uptake in lake chubsuckers (Erimyzon sucetta) exposed to polluted sediments. Archives of Environmental Contamination and Toxicology **39**:193-199.

Hopkins, W. A., B. P. Staub, J. W. Snodgrass, B. E. Taylor, A. E. DeBiase, J. H. Roe, B. P. Jackson, and J. D. Congdon. 2004. Responses of benthic fish exposed to contaminants in outdoor microcosms - examining the ecological relevance of previous laboratory toxicity tests. Aquatic Toxicology **68**:1-12.

Horowitz, A. J., M. Meybeck, Z. Idlafkih, and E. Biger. 1999. Variations in trace element geochemistry in the Seine River Basin based on floodplain deposits and bed sediments. Hydrological Processes **13**:1329-1340.

Jackson, B. P., P. L. S. Allen, W. A. Hopkins, and P. M. Bertsch. 2002. Trace element speciation in largemouth bass (Micropterus salmoides) from a fly ash settling basin by liquid chromatography-ICP-MS. Analytical and Bioanalytical Chemistry **374**:203-211.

Lemly, A. D. 1997. Ecosystem recovery following selenium contamination in a freshwater reservoir. Ecotoxicology and Environmental Safety **36**:275-281.

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

Lenat, D. R., and J. K. Crawford. 1994. Effects of land-use on water-quality and aquatic biota of 3 North-Carolina Piedmont streams. Hydrobiologia **294**:185-199.

Lesley, P., and P. N. Froelich. 2003. Arsenic, selenium, and antimony: from coal fired power plants to the Chattahoochee River. *in* K. J. Hatcher, editor. Proceedings of the 2003 Georgia Water Resources Conference. University of Georgia, Athens, GA.

Loeffler, G., J. L. Meyer, H. Trammell, and S. Holmbeck-Pelham. 2003. Fish consumption patterns along the Upper Chattahoochee River. *in* K. J. Hatcher, editor. Proceedings of the 2003 Georgia Water Resources Conference. University of Georgia, Athens, GA.

Lohner, T. W., R. J. Reash, V. E. Willet, and L. A. Rose. 2001a. Assessment of tolerant sunfish populations (Lepomis sp.) inhabiting selenium-laden coal ash effluents - 1. Hematological and population level assessment. Ecotoxicology and Environmental Safety **50**:203-216.

Lohner, T. W., R. J. Reash, and M. Williams. 2001b. Assessment of tolerant sunfish populations (Lepomis sp.) inhabiting selenium-laden coal ash effluents - 2. Tissue biochemistry evaluation. Ecotoxicology and Environmental Safety **50**:217-224.

Mason, R. P., J. M. Laporte, and S. Andres. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. Archives of Environmental Contamination and Toxicology **38**:283-297.

Meyer, J. L., M. J. Paul, and W. K. Taulbee. 2005. Stream ecosystem function in urbanizing landscapes. Journal of the North American Benthological Society **24**:602-612.

NRC. 2006. Managing coal combustion residues in mines. The National Academies Press, Washington, D.C.

Orr, P. L., K. R. Guiguer, and C. K. Russel. 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. Ecotoxicology and Environmental Safety **63**:175-188.

Paul, M. J., and J. L. Meyer. 2001. Streams in the urban landscape. Annual Review of Ecology and Systematics **32**:333-365.

Rosi-Marshall, E. J. 2002. Quality of suspended fine particulate matter and its role as a conduit for metals in riverine food webs. Ph.D. The University of Georgia, Athens.

Rowe, C. L., W. A. Hopkins, and J. D. Congdon. 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: A review. Environmental Monitoring and Assessment **80**:207-276.

Rowe, C. L., W. A. Hopkins, C. Zehnder, and J. D. Congdon. 2001. Metabolic costs incurred by crayfish (Procambarus acutus) in a trace element-polluted habitat: further evidence of similar responses among diverse taxonomic groups. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **129**:275-283.

Simmons, D. B. D., and D. Wallschlager. 2005. A critical review of the biogeochemistry and ecotoxicology of selenium in lotic and lentic environments. Environmental Toxicology and Chemistry **24**:1331-1343.

CHAPTER 2

USING TRACE ELEMENT CONCENTRATIONS IN *CORBICULA FLUMINEA* TO IDENTIFY POTENTIAL SOURCES OF CONTAMINATION IN AN URBAN RIVER[1]

---

[1]Peltier, Gretchen Loeffler, Judy L. Meyer, William A. Hopkins, Charles H. Jagoe, and Heather A. Brant. To be submitted to *Canadian Journal of Fisheries and Aquatic Sciences.*

Abstract

Rivers and streams in urban landscapes receive a multitude of anthropogenic inputs including urban/stormwater runoff, wastewater effluent, agricultural runoff, and effluent from coal-fired power plants. Previous studies found elevated metal concentrations in macroinvertebrates and fishes at all sites along a gradient of urbanization in the Chattahoochee River, which flows through Atlanta, Georgia. In this study we used the indicator species, *Corbicula fluminea*, to investigate the contributions of trace elements associated with different point sources and land uses in the Chattahoochee River basin. Trace elements were analyzed in tissues of clams collected from fifteen tributary streams draining five different land use or point source types: agriculture, forest, urban, coal-fired power plant (CFPP), and wastewater (WWTP). Concentrations of arsenic and selenium were highest (5.0 ± 0.2 and 13.6 ± 0.9 µg g^{-1} dry mass (DM), respectively) in clams from two CFPP sites. Cadmium concentrations were significantly higher in clams from urban and CFPP sites (4.1 ± 0.2 and 3.6 ± 0.9 µg g^{-1} DM, respectively). One forested site had the highest mercury concentration (0.56 ± 0.04 µg g^{-1} DM). Non-metric multidimensional scaling (NMS) of trace element concentrations in clams clustered CFPP sites and forest/agriculture sites at opposite ends of the ordination space. Cu, Zn, Cd, and Hg concentrations in clams were correlated with the two NMS axes. The elevated tissue concentrations of trace elements in *Corbicula fluminea* indicate the potential for elevated trace element concentrations in higher trophic levels of the food web in this urban river.

Introduction

Increased urbanization is associated with chemical, hydrological, and physical changes in rivers and streams. The term, urban stream syndrome, describes the patterns of chemical, physical, and biological change associated with increased urbanization in a catchment (Meyer et al. 2005). One component of the urban stream syndrome is elevated concentrations of metals (e.g. zinc, copper, cadmium) and metalloids (e.g. arsenic and selenium) which we collectively term trace elements, from various point and nonpoint sources including wastewater treatment plants, stormwater runoff, and industrial activities (Lenat and Crawford 1994, Horowitz et al. 1999, Paul and Meyer 2001). Elevated concentrations of copper, chromium, and lead in the water column were associated with urban land use (Lenat and Crawford 1994). Sediment-bound metals (e.g. copper, zinc, mercury) were also elevated in urban areas (Horowitz et al. 1999). During 1992-1995, the Chattahoochee River downstream of Atlanta, GA and urban tributaries to this river were among the most impacted sites with respect to all streams (urban, agriculture, and forest) evaluated by the National Water-Quality Assessment (NAWQA) program nationwide (Frick et al. 1998). In the Chattahoochee River, high concentrations of fecal coliform bacteria are often cited as the primary water quality concern (Rose 2002); yet, concentrations of lead and zinc are elevated in stream sediments in the metropolitan Atlanta area (Callender and Rice 2000), and macroinvertebrates and fishes in the Chattahoochee River had elevated concentrations of copper, arsenic, cadmium, and mercury (Rosi-Marshall 2002).

Point sources of pollution in urban catchments include combined sewer overflow, storm sewer outfalls, wastewater treatment plants (WWTPs), coal-fired power plants (CFPPs), and other industrial activities (Carpenter et al. 1998). Pharmaceuticals, personal care products, nitrogen, and phosphorus have been found in effluent from WWTPs (Daughton and Ternes 1999, Kolpin et al. 2002). Combined sewer overflow and WWTP effluent contain trace elements, and concentrations were correlated with WWTP discharge in an urban river as a result of shorter residence time in treatment during high flow events (Rozan and Benoit 2001, Karvelas et al.

2003). Aquatic disposal of coal combustion waste products is a significant source of trace element inputs into rivers and streams. Froelich and Lesley (2001) observed elevated dissolved and particulate concentrations of arsenic and selenium in the Chattahoochee River, but these concentrations quickly decreased downstream of two CFPP effluent discharges (Froelich and Lesley 2001). Knowledge of ecological effects of CFPP discharges in reservoirs is extensive (Hopkins et al. 2000, Lohner et al. 2001a, Lohner et al. 2001b, Rowe et al. 2001, Jackson et al. 2002, Lemly 2002, Rowe et al. 2002, Hopkins et al. 2004b); however, very little is known about the impacts of CFPP effluent to biota in lotic systems.

Nonpoint sources of pollution into rivers include urban and agricultural runoff, septic tank leachate, and atmospheric deposition (Carpenter et al. 1998). During baseflow, urban streams had higher concentrations of ions compared to non-urban streams, but dilution during stormflow reduced the difference between the two stream types (Rose 2002). Zinc concentrations were nearly an order magnitude higher in urban street runoff than runoff from shopping centers and suburban streets (Rose et al. 2001). Arsenic concentrations were elevated in soil and groundwater in cotton-producing regions, due to the use of arsenicals as herbicides and insecticides (Bednar et al. 2002). Elevated concentrations of arsenic, copper, and zinc have also been associated with poultry litter (Jackson and Bertsch 2001, Jackson et al. 2003). Atmospheric deposition of mercury occurs through precipitation and adsorption to aerosol particles reaching remote areas far from the original source (Fitzgerald et al. 1998, Morel et al. 1998).

This study was designed to determine if the type and extent of trace element contamination in the tissue of an indicator organism, *Corbicula fluminea*, varies with the type of point or nonpoint source in an urban river, which has elevated trace element content of aquatic insects and fishes (Rosi-Marshall 2002). The freshwater clam, *Corbicula fluminea*, was chosen to indicate the potential for contamination of the aquatic food web (see Chapter 4). We measured trace element concentrations in the whole body tissues of *Corbicula fluminea* from tributary and mainstem sites primarily in the Chattahoochee River basin, but also in the Broad River basin. The objectives of the study were to 1) Quantify trace element concentrations in *Corbicula* tissues downstream of two types of point and three types of nonpoint sources, and 2) Determine if the source type is correlated with tissue concentrations in the indicator organism. We hypothesize that clam tissues from sites receiving CFPP discharges (point source) will have elevated concentrations of arsenic, selenium, and cadmium, which are found in coal combustion wastes (Rowe et al. 2002). We predict that clams downstream of WWTP discharges (point source) will have elevated concentrations of zinc, cadmium, and lead (Karvelas et al. 2003). We also predict that clams from sites with highly urbanized catchments (nonpoint source) will have elevated concentrations of copper, zinc, and cadmium since these metals are associated with urban runoff (Horowitz et al. 1999, Rose et al. 2001, Rose 2002). Finally, we expect that clams from agricultural sites (nonpoint source) will have elevated concentrations of arsenic based on the history of cotton-farming in the region and prevalence of poultry operations (Jackson and Bertsch 2001, Bednar et al. 2002, Jackson et al. 2003).

Methods

Study sites

Fifteen tributary study sites were selected based on either the percentage of different land uses in the catchment or the predominant effluent source. The sites represented one of three predominant land use types or two types of effluent: forest, agriculture, or urban land use;

WWTP or CFPP effluent (Table 1). Fourteen sites were chosen in the Chattahoochee River basin and one forested site in the Broad River basin (Figure 2.1). We calculated land cover percentages using 1998 Landsat TM satellite imagery. Land cover for the study sites was characterized as percent urban (low and high density), agriculture (row crop and pasture), forest (deciduous, evergreen, and mixed), and other (golf course, clearcut, open water). The percent land use was determined for the entire catchment upstream of the study site.

We surveyed six streams with forested catchments in the Chattahoochee River basin. Only two had *Corbicula fluminea*, so the third forested site was located in the Broad River basin. Catchment area for forested sites ranged from 64 – 1761 km^2, which encompassed the range of watershed sizes for the other land use categories (Table 2.1).

The three agricultural tributaries empty into Lake Lanier, an impoundment on the Chattahoochee River. These watersheds were farmed for cotton in the 1800s; today poultry farms dominate the landscape. Catchment area ranged from 43 - 68 km^2, and agricultural land use ranged from 25 – 33% (Table 2.1). Forested land cover was high, ranging from 50 – 64%, and in these catchments agricultural land use is primarily pasture that is used for disposal of poultry waste and cattle grazing, which is typical of agricultural watersheds in the basin.

The three urban streams were located within metropolitan Atlanta. The population density in the Nancy Creek and Peachtree Creek stream network was approximately 2400 people/km^2 in 2000 (Rose et al. 2001). Population density in the Sope Creek catchment was 800 people/km^2 (Meyer et al. 2005). These streams have flashy hydrographs and frequently overflow their banks during rain events. Catchment area ranged from 69 – 223 km^2 and urban land use ranged from 65 – 80% (Table 2.1).

The three CFPP sites were located in the mainstem of the Chattahoochee River, immediately downstream of the effluent discharge. Plant McDonough and Plant Wansley have one settling basin and Plant Yates has two ponds. Two of the plants discharge directly into the river and the third (Plant Wansley) discharges into Yellowdirt Creek just before its confluence with the Chattahoochee River. Discharges vary to maintain pond level in the settling basins. Energy generation capacity ranged from 490 – 1730 megawatts (Table 2.1).

We surveyed six streams receiving discharges from WWTPs; *Corbicula fluminea* were found in only three of them. The sampling sites in these streams were at the first access point downstream of the effluent discharge. Catchment area ranged from 76 – 268 km^2 (Table 2.1). NPDES permit limits for the plants ranged from 2 – 24 million gallons per day (MGD).

Water quality

Each site was sampled once during once April-August 2004. Temperature, specific conductance, pH, and dissolved oxygen were measured with a YSI sonde (Yellow Springs, OH). Whole water samples were collected in acid-washed polyethylene bottles and filtered in the field with pre-ashed (1 hour at 500°F) glass fiber filters (Whatman GFF). We measured total suspended solids by drying filters at 60°C for 1 week and then weighing. Filtrate was retained for analysis of dissolved organic carbon (DOC), ammonium (NH_4^+), nitrate (NO_3^-), total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), cations, and anions. Concentrations of TDN and TDP were determined using the methods of Wetzel and Likens (2000). DOC was measured using a total carbon analyzer (Sievers Model 800 Turbo, Boulder, CO).

Cations (Ca^{2+}, K^+, Mg^{2+}, NH_4^+, Na^+) and anions (SO_4^{2-}, Cl^-, NO_3^-) were analyzed by ion chromatography using a Metrohm-Peak (Houston, TX) Metrosep C 2 - 100 resin column (cations) and Metrosep A Supp 5 - 100 resin columns with chemical suppression (anions). Samples (150 µl) were injected and analyzed for a minimum of 3 repetitions, with any outliers rejected. Concentrations were measured by conductimetry and chromatography peak areas were manually integrated. Cation and anion concentrations can be found in Appendix A.1.

Trace element sampling and analysis

In the field, unfiltered water was collected in acid-washed polyethylene bottles for total trace element concentrations. Separate water samples were filtered in the field using GHP Acrodisc GF 25 mm syringe filters with GF/0.45 µm GHP membranes (Pall Life Sciences, East Hills, NY, USA) for dissolved trace element concentrations. Both filtered and unfiltered trace element water samples were acidified with trace-metal grade nitric acid prior to freezing. Field blanks using deionized-distilled water were treated in the same manner. Unfiltered water samples (45 ml) were digested with 5 ml of trace metal grade nitric acid in a microwave (CEM Corporations, Matthews, NC, USA). Analysis of filtered and unfiltered samples is described below. Particulate fractions of trace elements in the water column were calculated by subtracting the concentration in filtered samples (dissolved) from the unfiltered samples.

We collected approximately 15 clams of similar size (15-20 mm total length) from each site. Clams were kept in aerated site water for 24 hours for gut depuration, placed in plastic bags, and frozen. After thawing, clams were measured for length and wet total and tissue weight; soft tissues were then removed and frozen in sterile microcentrifuge tubes, freeze-dried, and dry tissue mass (DM) recorded.

Tissue from nine similarly-sized individuals (18.40 ± 0.13 mm length) from each site was analyzed for trace element concentrations. We chose to analyze a subset of nine individuals to minimize effects of variation in size among sites and cost of analysis. Approximately 20-60 mg

DM of clam tissue was used for digestion. Trace metal grade nitric acid (2.5 ml) was added to the samples prior to digestion in a microwave (CEM Corporation, Matthews, NC, USA) with heating steps of 60, 60, 70, 80% microwave power for 10, 10, 15, and 20 minutes, respectively. After digestion, 0.5 ml of trace metal grade hydrogen peroxide was added to the samples and microwaved at the same power and duration as the first digestion. Samples were brought to a final volume of 10 ml with high-purity deionized water. All trace element analyses were performed using an inductively coupled plasma mass spectrometer (ICP-MS) (Perkin Elmer, Norwalk, CT). We measured total concentrations of vanadium (V), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), cadmium (Cd), mercury (Hg), and lead (Pb). Concentrations of V, Mn, Fe, Co, Sr are not reported in this chapter, but all trace element concentrations for tissues and water are presented in Appendices A.2 and A.3. Certified reference materials of appropriate matrix (Riverwater and Tort 2; NRC, Ottawa, Canada) and blanks were included in the digestion and analysis procedures for QA/QC purposes. Mean percent recoveries for trace elements in tissues and water reference material ranged from 89 – 125%.

Statistical analyses

All tissue concentrations are reported as means for each site (N=9) with standard errors. We tested the normality of trace element concentrations at each site with the Shapiro-Wilk's test, and transformed them using $\log_{10}(x+1)$ when necessary. Shell lengths of clams were compared with a one-way ANOVA followed by Tukey-Kramer post hoc tests. We intended to compare means of trace elements among site types (e.g., agriculture, urban). However, sites within a site type differed significantly in trace element concentrations, so all comparisons were among fifteen sites rather than five site types. Multivariate analysis of variance (MANOVA) was used to test for differences in log-transformed trace element concentrations among the fifteen study sites. Elements that were significantly different among the fifteen sites were subsequently analyzed with one-way ANOVAs followed by Tukey-Kramer post hoc tests. Significant differences were reported at the $p < 0.05$ level of confidence. Pearson's correlation coefficient (r) was used to examine relationships among log-transformed trace element concentrations across all study sites. JMP 5.0.1 or SAS 9.1 (SAS Institute, Cary, NC, USA) were used for statistical analyses.

Overall variation in trace element tissue concentrations among the study sites was examined using non-metric multidimensional scaling (NMS) ordination with Sorenson distance measure using the statistical package PC-ORD (MJM Software Design, Glenden Beach, OR, USA. NMS uses an iterative search for low stress (< 10 is ideal). Stress is measured as the relationship between ranked distances in the original multi-dimensional space and ranked distances in the reduced dimensions of the ordination. NMS is commonly used to compare ecological community composition across multiple sites (Roy et al. 2003, Dodson et al. 2005). We used this indirect ordination technique to determine site similarity based on overall trace element concentrations in *Corbicula fluminea* tissue. We used mean trace element concentrations of Ni, Cu, Zn, As, Se, Sr, Cd, Hg, and Pb in the tissues of clams from each study site to position sites in ordination space. Correlation coefficients of NMS axes with measured environmental or land cover variables and with individual trace elements were calculated to determine factors associated with each axis.

Results

Water quality

All study sites were sampled during baseflow except Nancy Creek and Broad River. Nancy Creek was sampled during the rising limb of the hydrograph and Broad River was sampled several days after a large rain event. Total suspended solids concentration was highest at Nancy Creek, likely due to the precipitation (Table 2.2). Other water quality parameters (pH and temperature) did not vary significantly among sites. Specific conductance differed among sites, and the highest values were from CFPP and urban site types whereas forested site types had the lowest values (Table 2). Dissolved organic carbon (DOC) ranged between 2.01-12.10 mg/L with the highest concentrations at the CFPP and urban site types. However, regressions between mean tissue concentration of trace elements and DOC concentration at each site were not significant. Nutrient concentrations ranged widely among the study sites. TDP and TDN concentrations were lowest at Flat Shoals Creek (forest) respectively (Table 2.2). West Fork Little River (forest) and Nancy Creek (urban) had the highest TDN and TDP concentrations, respectively (Table 2.2).

Dissolved concentrations of trace elements in the water column varied among study sites (Appendix A.3). The East Fork Little River (agriculture) site had the lowest dissolved concentrations of Zn, As, and Se at 22, 0.12, 0.11 μg/L respectively. The Plant Yates (CFPP) site had the highest dissolved concentrations of Ni, As, and Se. We chose to not use the particulate fraction concentrations because the values were negative for some trace elements. Specifically, all of Zn and Cd particulate concentrations and 20-30% of Ni, Cu, and Se particulate concentrations were negative. Incomplete digestion of unfiltered water samples likely contributed to this problem.

Clams

Clam lengths were not significantly different among forest, agriculture, urban, and CFPP sites (ANOVA, $p > 0.05$). Clams from wastewater treatment plant sites were significantly larger; however their lengths remained within the range of 15 - 20 mm (Appendix A.4). Regressions between trace element concentrations and length were significant ($p < 0.05$) only for Ni and Cd (Figure 2.2). However, length only explained 3-9% of the variance in trace element concentration. Therefore size differences among clams are not a likely explanation for differences in trace element concentrations observed among sites.

Sites (N=9) within a site type (e.g., forest, agriculture) had significantly different concentrations of trace elements so we were unable to pool sites by type. Although we could have used site means to compare among site types, the relatively low replication (N=3 in each site type) results in low statistical power. Instead we analyzed differences in trace element concentrations in clams among the fifteen study sites and focused on the sites that had highest and lowest concentrations.

Trace element element concentrations differed significantly among the study sites (MANOVA, $p<0.05$). Rather than discussing how each site differs, we focus on sites with concentrations of Ni, Cu, Zn, As, Se, Cd, Hg, and Pb that are either significantly higher or lower than other sites. Of the fifteen study sites, clams from nine sites had significantly higher/lower concentrations of Ni, Cu, Zn, As, Se, Cd, Hg, and Pb. Trace element concentrations in clams from the other six study sites were in the middle of the concentration range.

Clams from Flat Shoals Creek (forest) and Plant Wansley (CFPP) had higher concentrations of Ni whereas the lowest concentrations were found at an agricultural site, East Fork Little River (Figure 2.3). Mean tissue concentrations of Cu were higher at the Plant Yates

(CFPP), Peachtree Creek (urban), and Plant McDonough (CFPP) sites, and Cu concentrations were significantly lower at all other sites (Figure 2.4). Significantly higher concentrations of Zn were found in clam tissues from the Plant McDonough (CFPP), Plant Wansley (CFPP), and Peachtree Creek (urban) sites, whereas the lowest concentrations were in clams from the Broad River (forest) site (Figure 2.5). Mean As concentrations in tissues were highest at the Plant Wansley (CFPP) site and lowest at the East Fork Little River (agriculture) site (Figure 2.6). Se concentrations were significantly higher in clams from the two CFPP sites (Plant Wansley and Plant Yates), and the lowest concentrations were found in those from a WWTP site (Anneewakee Creek) (Figure 2.6). The highest concentrations of Cd in clams were found at the Peachtree Creek (urban) site (Figure 2.3). Hg concentrations were significantly higher at one of the forested sites, Flat Shoals Creek whereas the highest Pb concentrations were found in clams from a WWTP site (Big Creek) (Figure 2.7).

Relationship to land cover

We compared tissue concentrations of all trace elements at each study site with percentage of urban, forest, and agricultural land cover at that site. Mean tissue concentrations of Hg were positively correlated with percent forested land cover. In contrast, agricultural land cover was negatively correlated with mean tissue concentrations of Ni. Percent urban land cover was not significantly (positively or negatively) related to tissue concentrations of any of the trace elements reported here.

Correlations among trace elements

Several of the trace element concentrations in tissues of individual clams were highly correlated across study sites (Table 2.3). We found strong positive correlations between Cu, Zn, As, Se, and Cd (Table 2.3). Tissue concentrations of Hg were not highly correlated with any of the trace elements reported here. Tissue concentrations were not correlated with dissolved concentrations of any trace element.

NMS ordination

A two-dimensional ordination of the fifteen sites defined by nine trace elements was recommended from comparison of real and randomized data in NMS. The best NMS solution had a stress value of 0.958. Axis 1 accounted for 91% of the variation and axis 2 contributed an additional 8.5% for a total of 99.5% of variance accounted for by the ordination. Two forest sites, two agriculture sites, and one WWTP site (Annewakee Creek) grouped together on the forest/agriculture side of ordination space. All three CFPP sites and one urban site (Peachtree Creek) clustered together on the opposite end of the ordination space. Two environmental variables (DOC and conductivity) and three land cover variables (%urban, %agriculture, and %forest) were correlated ($r^2 > 0.30$) with the grouping of sites (Figure 2.8). DOC, conductivity, %urban, and % agriculture were correlated ($r^2 > 0.30$) with axis 1 whereas %urban and %forest were negatively correlated with axis 2 ($r^2 > 0.40$) (Table 2.4). Cu, Zn, and Cd concentrations in clams were positively correlated with axis 1 and Hg concentrations were positively correlated with axis 2 (Table 2.4).

Discussion

Site characterization

We were unable to collapse the study sites into site types because trace element concentrations were significantly different among study sites within site types (e.g., forest). The patterns we saw are evidence of the inherent variability in water chemistry, biological activity, and geomorphology in streams across the landscape. In addition, historical differences in land use, efficiency of instream processes, and supply of pollutants leads to variability in the chemistry of urban streams (Walsh et al. 2005). Historical legacy also influences forested catchments since extensive row-crop agriculture dominated the region until the 1920s when forested areas returned (Roy et al. 2003). In the southern Appalachians, stream invertebrate assemblages reflected agricultural land use 50 years earlier (Harding et al. 1998). Streams can be categorized based upon land cover or catchment area, but streams within these categories still vary considerably. Assuming an "urban" stream in one catchment will function in a similar manner as another "urban" stream in a neighboring catchment ignores the uniqueness and variability just described.

Trends in trace element concentrations

Despite the differences within site type categories, we were able to draw conclusions about overall trends in trace element concentrations. Clams from the reference (forested) sites had the highest tissue concentrations of Hg. Natural and anthropogenically derived Hg is primarily distributed through atmospheric deposition (Mason et al. 1994, Fitzgerald et al. 1998, Morel et al. 1998). Forests are efficient in collecting Hg-containing aerosols and particulates, which may later be washed out during precipitation events (Kolka et al. 1999a, Kolka et al. 1999b, Grigal et al. 2000). Other studies have shown higher concentrations of Hg associated with forested land cover (Sonesten 2001, 2003). Hg concentrations in fish (*Perca fluviatilis* and *Rutilus rutilus*) were higher in lakes with forested catchments; higher humic acids and DOC likely contributed to this relationship (Sonesten 2001, 2003). The percentage of forested land cover in the agricultural sites was similar to forest cover in the forested catchments, and clams from agricultural sites also had higher concentrations of Hg when compared with the other site types. In the Albemarle-Pamlico drainage basin, tissue concentrations of Hg in *Corbicula fluminea* from highly forested catchments ranged between 0.02 to 0.22 $\mu g\ g^{-1}$ WM (0.08 to 0.88

μg g^{-1} DM, assuming 75% moisture content) (Ruhl and Smith 1996) compared to 0.13 to 0.56 μg g^{-1} DM from forested and agricultural catchments in this study. Hence clams from Chattahoochee River forested and agricultural tributaries had similar mercury concentrations in their tissue as clams from other southeastern forested tributaries.

Clams from the Peachtree Creek (urban) site had higher tissue concentrations of Cd, Cu, and Zn. All of these metals are associated with urban runoff from impervious surfaces. Dissolved concentrations of Cd, Cu, and Zn, which would vary with baseflow or stormflow conditions, were lower at Peachtree Creek (base) and higher at Nancy Creek (storm). *Corbicula fluminea* are filter-feeders, and their tissue concentrations reflect trace element concentrations in the water and sediments over time rather than simply at the time of sampling. Clams integrate exposure to contaminants, which explains why concentrations of Cd, Cu, and Zn were higher in clams from Peachtree Creek even though dissolved concentrations of these elements were not elevated at the time of sampling. In a previous study, median dissolved concentrations of Zn and Cu in Peachtree Creek during baseflow were 14 and 2.7 μg/L, respectively, compared with 60 and 1.9 μg/L during stormflow (Rose et al. 2001). These concentrations are an order of magnitude lower than concentrations measured in urban street runoff, which implies dilution of pulses once they enter urban tributaries (Rose et al. 2001).

We predicted that clams downstream of the three CFPPs would have elevated tissue concentrations of trace elements, and these predictions were supported. CFPP discharges in the Chattahoochee River were associated with higher concentrations of As and Se in the tissues of *Corbicula fluminea*. Clams from the Plant Wansley (CFPP) site had the highest concentrations of Zn, As, and Se (Figures 2.5 and 2.6). Of the three CFPP sites, Plant McDonough had the highest concentrations of Cu in clams (Figure 2.4). All three plants burn eastern bituminous coal so the differences among plants are not likely related to the type of coal burned. Plants McDonough and Yates also burn up to 20% natural gas to meet NO_x emission targets during the summer months. However, the differences in trace element concentrations may simply be related to energy generation capacity (Wansley > Yates > McDonough, Table 2.1) rather than specific combustion processes. As and Ni concentrations in clams from CFPP sites follow the pattern of energy generation capacity, and Se concentrations were lower at McDonough site compared to Plants Wansley and Yates. For example, the Se concentrations (9.8 and 13.6 $\mu g\ g^{-1}$ DM) in clams from two CFPP sites in the Chattahoochee River were higher than Se concentrations (8.7 $\mu g\ g^{-1}$ DM) found in clams in the ash basins of a small CFPP with lower energy generation capacity in South Carolina (Nagle et al. 2001). However, mean As and Cd concentrations from the two CFPP Chattahoochee sites were lower than As and Cd concentrations (13 and 4 $\mu g\ g^{-1}$ DM, respectively) in the South Carolina study (Nagle et al. 2001).

NMS ordination

NMS ordination of sites based on trace element concentrations was consistent with the observation that nine of the fifteen sites had significantly higher/lower trace element concentrations (Figure 2.8). All three CFPP sites and one urban site (Peachtree Creek) grouped together along the ordination axis correlated with conductivity and DOC. High concentrations of Zn, Cu, and Cd in clam tissues characterized those four sites (Table 2.4). As discussed earlier, forested and agricultural sites had similar percentages of forested land cover in their catchment, and forested catchments were probably agricultural land a century ago. Thus, it is not surprising that two of the agricultural and two of the forested catchments cluster together in the NMS ordination. Higher concentrations of Hg characterized those agricultural and forested sites

(Table 2.4). Anneewakee Creek (WWTP) also clusters with the four forest/agriculture sites, which may reflect the high percentage of forested land cover in its catchment (Table 2.1). The other two WWTP sites were located closer to downtown Atlanta than Anneewakee Creek, but the % urban cover was similar among the three WWTP sites (Table 2.1). The six sites (two WWTP, two urban, one forested, and one agricultural) where trace element concentrations exhibited intermediate concentrations (Figure 2.5) were located in the center of the NMS ordination (Figure 2.8).

Conclusion

Tissue concentrations of trace elements in an indicator organism, *Corbicula fluminea*, can be related to point and nonpoint sources in the stream's catchment. Clams from highly forested catchments had higher Hg concentrations in their tissues, and clams from CFPP sites had consistently higher concentrations of several other trace elements (especially Cu, Zn, As, Se, and Cd). Clams from some highly urbanized tributaries also had elevated trace element concentrations. Inputs from CFPPs and urbanized tributaries are contributing trace elements to the Chattahoochee River. The tissue concentrations of trace elements in *Corbicula fluminea* indicate the potential for elevated concentrations of trace elements in higher trophic levels in these aquatic food webs. Further evaluation of the extent of trace element contamination in the food webs of this river is warranted.

Acknowledgements

The authors would like to thank Brian P. Jackson and the Advanced Analytical Center for Environmental Sciences at the Savannah River Ecology Laboratory for assistance in ICP-MS analysis. Rick Peltier analyzed the cations and anions at the Georgia Institute of Technology. We would like to thank Erica Chiao, Krista Jones, John Kominoski, Rick Peltier, and Lindsay Stallcup for assistance with field collections of clams. Special thanks to Harlan Trammell, Birgit Bolton, and the Upper Chattahoochee Riverkeeper for their assistance in field sampling and boat access to the mainstem sites.

Literature Cited

Bednar, A. J., J. R. Garbarino, J. F. Ranville, and T. R. Wildeman. 2002. Presence of organoarsenicals used in cotton production in agricultural water and soil of the southern United States. Journal of Agricultural and Food Chemistry **50**:7340-7344.

Beltman, D. J., W. H. Clements, J. Lipton, and D. Cacela. 1999. Benthic invertebrate metals exposure, accumulation, and community-level effects downstream from a hard-rock mine site. Environmental Toxicology and Chemistry **18**:299-307.

Besser, J. M., W. G. Brumbaugh, T. W. May, S. E. Church, and B. A. Kimball. 2001. Bioavailability of metals in stream food webs and hazards to brook trout (Salvelinus fontinalis) in the upper Animas River watershed, Colorado. Archives of Environmental Contamination and Toxicology **40**:48-59.

Callender, E., and K. C. Rice. 2000. The urban environmental gradient: Anthropogenic influences on the spatial and temporal distributions of lead and zinc in sediments. Environmental Science & Technology **34**:232-238.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications **8**:559-568.

Clements, W. H. 1994. Benthic invertebrate community responses to heavy-metals in the Upper Arkansas River basin, Colorado. Journal of the North American Benthological Society **13**:30-44.

Clements, W. H., D. M. Carlisle, J. M. Lazorchak, and P. C. Johnson. 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecological Applications **10**:626-638.

Clements, W. H., and P. M. Kiffney. 1995. The influence of elevation on benthic community responses to heavy-metals in Rocky-Mountain streams. Canadian Journal of Fisheries and Aquatic Sciences **52**:1966-1977.

Cohen, J. E. 2003. Human population: The next half century. Science **302**:1172-1175.

Crutchfield, J. U. 2000. Recovery of a power plant cooling reservoir ecosystem from selenium bioaccumulation. Environmental Science & Policy **3**:145-163.

Daughton, C. G., and T. A. Ternes. 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environmental Health Perspectives **107**:907-938.

Dodson, S. I., R. A. Lillie, and S. Will-Wolf. 2005. Land use, water chemistry, aquatic vegetation, and zooplankton community structure of shallow lakes. Ecological Applications **15**:1191-1198.

Farag, A. M., D. F. Woodward, J. N. Goldstein, W. Brumbaugh, and J. S. Meyer. 1998. Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River Basin, Idaho. Archives of Environmental Contamination and Toxicology **34**:119-127.

Fitzgerald, W. F., D. R. Engstrom, R. P. Mason, and E. A. Nater. 1998. The case for atmospheric mercury contamination in remote areas. Environmental Science & Technology **32**:1-7.

Frick, E. A., D. J. Hippe, G. R. Buell, C. A. Couch, E. H. Hopkins, D. J. Wangsness, and J. W. Garrett. 1998. Water quality in the Apalachicola-Chattahoochee-Flint River Basin, Georgia, Alabama, and Florida, 1992-1995. USGS Circular Report 1164.

Froelich, P. N., and P. Lesley. 2001. Tracing germanium contamination from coal-fired power plants along the Chattahoochee River-Apalachicola River: implications for the toxic metalloids arsenic and selenium. *in* K. J. Hatcher, editor. Proceedings of the Georgia Water Resources Conference. Institute of Ecology, University of Georgia, Athens, GA.

Grigal, D. F., R. K. Kolka, J. A. Fleck, and E. A. Nater. 2000. Mercury budget of an upland-peatland watershed. Biogeochemistry **50**:95-109.

Guthrie, R. K., and D. S. Cherry. 1979. Trophic level accumulation of heavy-metals in a coal ash basin drainage system. Water Resources Bulletin **15**:244-248.

Hamilton, S. J. 2002. Rationale for a tissue-based selenium criterion for aquatic life. Aquatic Toxicology **57**:85-100.

Hamilton, S. J. 2004. Review of selenium toxicity in the aquatic food chain. Science of the Total Environment **326**:1-31.

Hamilton, S. J., and K. J. Buhl. 2004. Selenium in water, sediment, plants, invertebrates, and fish in the blackfoot river drainage. Water Air and Soil Pollution **159**:2-34.

Harding, J. S., E. F. Benfield, P. V. Bolstad, G. S. Helfman, and E. B. D. Jones. 1998. Stream biodiversity: The ghost of land use past. Proceedings of the National Academy of Sciences of the United States of America **95**:14843-14847.

Hopkins, W. A., J. W. Snodgrass, J. H. Roe, B. P. Jackson, J. C. Gariboldi, and J. D. Congdon. 2000. Detrimental effects associated with trace element uptake in lake chubsuckers

(Erimyzon sucetta) exposed to polluted sediments. Archives of Environmental Contamination and Toxicology **39**:193-199.

Hopkins, W. A., B. P. Staub, J. W. Snodgrass, B. E. Taylor, A. E. DeBiase, J. H. Roe, B. P. Jackson, and J. D. Congdon. 2004. Responses of benthic fish exposed to contaminants in outdoor microcosms - examining the ecological relevance of previous laboratory toxicity tests. Aquatic Toxicology **68**:1-12.

Horowitz, A. J., M. Meybeck, Z. Idlafkih, and E. Biger. 1999. Variations in trace element geochemistry in the Seine River Basin based on floodplain deposits and bed sediments. Hydrological Processes **13**:1329-1340.

Jackson, B. P., P. L. S. Allen, W. A. Hopkins, and P. M. Bertsch. 2002. Trace element speciation in largemouth bass (Micropterus salmoides) from a fly ash settling basin by liquid chromatography-ICP-MS. Analytical and Bioanalytical Chemistry **374**:203-211.

Jackson, B. P., and P. M. Bertsch. 2001. Determination of arsenic speciation in poultry wastes by IC-ICP-MS. Environmental Science & Technology **35**:4868-4873.

Jackson, B. P., P. M. Bertsch, M. L. Cabrera, J. J. Camberato, J. C. Seaman, and C. W. Wood. 2003. Trace element speciation in poultry litter. Journal of Environmental Quality **32**:535-540.

Karvelas, M., A. Katsoyiannis, and C. Samara. 2003. Occurrence and fate of heavy metals in the wastewater treatment process. Chemosphere **53**:1201-1210.

Kolka, R. K., D. F. Grigal, E. S. Verry, and E. A. Nater. 1999a. Mercury and organic carbon relationships in streams draining forested upland peatland watersheds. Journal of Environmental Quality **28**:766-775.

Kolka, R. K., E. A. Nater, D. F. Grigal, and E. S. Verry. 1999b. Atmospheric inputs of mercury and organic carbon into a forested upland bog watershed. Water Air and Soil Pollution **113**:273-294.

Kolpin, D. W., E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber, and H. T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. Environmental Science & Technology **36**:1202-1211.

Lemly, A. D. 1985. Toxicology of selenium in a fresh-water reservoir - Implications for environmental-hazard evaluation and safety. Ecotoxicology and Environmental Safety **10**:314-338.

Lemly, A. D. 1993. Teratogenic effects of selenium in natural-populations of fresh-water fish. Ecotoxicology and Environmental Safety **26**:181-204.

Lemly, A. D. 1997. Ecosystem recovery following selenium contamination in a freshwater reservoir. Ecotoxicology and Environmental Safety **36**:275-281.

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

Lenat, D. R., and J. K. Crawford. 1994. Effects of land-use on water-quality and aquatic biota of 3 North-Carolina Piedmont streams. Hydrobiologia **294**:185-199.

Lesley, P., and P. N. Froelich. 2003. Arsenic, selenium, and antimony: from coal fired power plants to the Chattahoochee River. *in* K. J. Hatcher, editor. Proceedings of the 2003 Georgia Water Resources Conference. University of Georgia, Athens, GA.

Loeffler, G., J. L. Meyer, H. Trammell, and S. Holmbeck-Pelham. 2003. Fish consumption patterns along the Upper Chattahoochee River. *in* K. J. Hatcher, editor. Proceedings of the 2003 Georgia Water Resources Conference. University of Georgia, Athens, GA.

Lohner, T. W., R. J. Reash, V. E. Willet, and L. A. Rose. 2001a. Assessment of tolerant sunfish populations (Lepomis sp.) inhabiting selenium-laden coal ash effluents - 1. Hematological and population level assessment. Ecotoxicology and Environmental Safety **50**:203-216.

Lohner, T. W., R. J. Reash, and M. Williams. 2001b. Assessment of tolerant sunfish populations (Lepomis sp.) inhabiting selenium-laden coal ash effluents - 2. Tissue biochemistry evaluation. Ecotoxicology and Environmental Safety **50**:217-224.

Luoma, S. N., C. Johns, N. S. Fisher, N. A. Steinberg, R. S. Oremland, and J. R. Reinfelder. 1992. Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. Environmental Science & Technology **26**:485-491.

Luoma, S. N., and P. S. Rainbow. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environmental Science & Technology **39**:1921-1931.

Mason, R. P., W. F. Fitzgerald, and F. M. M. Morel. 1994. The biogeochemical cycling of elemental mercury - Anthropogenic influences. Geochimica Et Cosmochimica Acta **58**:3191-3198.

Mason, R. P., J. M. Laporte, and S. Andres. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. Archives of Environmental Contamination and Toxicology **38**:283-297.

Meyer, J. L., M. J. Paul, and W. K. Taulbee. 2005. Stream ecosystem function in urbanizing landscapes. Journal of the North American Benthological Society **24**:602-612.

Morel, F. M. M., A. M. L. Kraepiel, and M. Amyot. 1998. The chemical cycle and bioaccumulation of mercury. Annual Review of Ecology and Systematics **29**:543-566.

Nagle, R. D., C. L. Rowe, and J. D. Congdon. 2001. Accumulation and selective maternal transfer of contaminants in the turtle Trachemys scripta associated with coal ash deposition. Archives of Environmental Contamination and Toxicology **40**:531-536.

NRC. 2006. Managing coal combustion residues in mines. The National Academies Press, Washington, D.C.

Orr, P. L., K. R. Guiguer, and C. K. Russel. 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. Ecotoxicology and Environmental Safety **63**:175-188.

Paul, M. J., and J. L. Meyer. 2001. Streams in the urban landscape. Annual Review of Ecology and Systematics **32**:333-365.

Pedlar, R. M., M. D. Ptashynski, K. G. Wautier, R. E. Evans, C. L. Baron, and J. F. Klaverkamp. 2002. The accumulation, distribution, and toxicological effects of dietary arsenic exposure in lake whitefish (Coregonus clupeaformis) and lake trout (Salvelinus namaycush). Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **131**:73-91.

Rose, S. 2002. Comparative major ion geochemistry of Piedmont streams in the Atlanta, Georgia region: possible effects of urbanization. Environmental Geology **42**:102-113.

Rose, S., M. S. Crean, D. K. Sheheen, and A. M. Ghazi. 2001. Comparative zinc dynamics in Atlanta metropolitan region stream and street runoff. Environmental Geology **40**:983-992.

Rosi-Marshall, E. J. 2002. Quality of suspended fine particulate matter and its role as a conduit for metals in riverine food webs. Ph.D. The University of Georgia, Athens.

Rowe, C. L., W. A. Hopkins, and J. D. Congdon. 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: A review. Environmental Monitoring and Assessment **80**:207-276.

Rowe, C. L., W. A. Hopkins, C. Zehnder, and J. D. Congdon. 2001. Metabolic costs incurred by crayfish (Procambarus acutus) in a trace element-polluted habitat: further evidence of similar responses among diverse taxonomic groups. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **129**:275-283.

Roy, A. H., A. D. Rosemond, M. J. Paul, D. S. Leigh, and J. B. Wallace. 2003. Stream macroinvertebrate response to catchment urbanisation (Georgia, USA). Freshwater Biology **48**:329-346.

Rozan, T. F., and G. Benoit. 2001. Mass balance of heavy metals in New Haven Harbor, Connecticut: Predominance of nonpoint sources. Limnology and Oceanography **46**:2032-2049.

Ruhl, P. M., and K. E. Smith. 1996. Water-quality assessment of the Albemarle-Pamlico drainage basin, North Carolina and Virginia - Trace elements in Asiatic clam (*Corbicula fluminea*) soft tissues and redbreast sunfish (*Lepomis auritus*) livers, 1992-93. Open-File Report 96-334.

Simmons, D. B. D., and D. Wallschlager. 2005. A critical review of the biogeochemistry and ecotoxicology of selenium in lotic and lentic environments. Environmental Toxicology and Chemistry **24**:1331-1343.

Sonesten, L. 2001. Mercury content in roach (Rutilus rutilus L.) in circumneutral lakes - effects of catchment area and water chemistry. Environmental Pollution **112**:471-481.

Sonesten, L. 2003. Catchment area composition and water chemistry heavily affects mercury levels in perch (Perca fluviatilis L.) in circumneutral lakes. Water Air and Soil Pollution **144**:117-139.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P. Morgan. 2005. The urban stream syndrome: current knowledge and the search for a cure. Journal of the North American Benthological Society **24**:706-723.

Table 2.1. Catchment area and land cover percentages for all fifteen study sites. Land coverage determined from 1998 Landsat TM imagery. [1]Units for point sources are megawatts (coal) and million gallons per day (wastewater).

Site Name	Site Type	Point Source[1]	Watershed area (km^2)	Urban	Agriculture	Forest	Other
Broad River	Forest	-	1761.4	8.1%	24.3%	58.2%	9.4%
Flat Shoals Creek	Forest	-	113.2	3.6%	6.5%	81.6%	8.3%
Snake Creek	Forest	-	63.6	8.1%	10.0%	70.9%	10.9%
East Fork Little River	Agriculture	-	43.0	8.8%	33.0%	50.5%	7.7%
Wahoo Creek	Agriculture	-	63.7	7.9%	24.8%	64.3%	3.0%
West Fork Little River	Agriculture	-	47.4	9.5%	31.5%	51.1%	7.9%
Nancy Creek	Urban	-	69.2	73.8%	0.5%	22.7%	3.0%
Peachtree Creek	Urban	-	223.0	79.7%	0.4%	18.7%	1.2%
Sope Creek	Urban	-	79.7	64.6%	0.9%	32.1%	2.5%
Plant McDonough	Coal	490	4120.5	26.1%	10.7%	53.8%	9.4%
Plant Wansley	Coal	1730	6620.5	25.3%	10.2%	56.6%	8.0%
Plant Yates	Coal	1250	6264.7	26.0%	10.0%	56.2%	7.8%
Anneewakee Creek	Wastewater	3.25	76.3	36.8%	3.6%	55.5%	4.0%
Big Creek	Wastewater	24	268.4	35.2%	13.0%	46.2%	5.6%
Suwanee Creek	Wastewater	2	109.3	33.2%	8.2%	51.2%	7.4%

Table 2.2. Water quality parameters and sampling dates for all fifteen study sites. Blanks indicate no data was collected.

Site Name	Site Type	Date	Temperature (°C)	Specific conductance (mS/cm)	pH	DOC (mg/L)	TSS (mg/L)	Total P (µg/L)	Total N (mg/L)
Broad River	Forest	6/28/2004	22.88	0.052	7.15	4.73	4.6	78.20	0.83
Flat Shoals Creek	Forest	9/6/2004	22.67	0.051	7.5	2.29	56.42	5.40	0.30
Snake Creek	Forest	6/18/2004	22.58	0.033	7.36	2.19	9.79	60.20	0.35
East Fork Little River	Agriculture	4/2/2004	10.48	0.114	7.83	3.92	2.62	34.40	1.10
Wahoo Creek	Agriculture	9/6/2004	21.47	0.063	7.61	2.01	8.5	19.40	0.62
West Fork Little River	Agriculture	3/23/2004	6.56	0.141	7.9	3.86	3.33	27.90	2.06
Nancy Creek	Urban	4/26/2004	13.89	0.344	7.5	7.38	130.5	107.90	1.00
Peachtree Creek	Urban	3/18/2004	14.05	0.225	7.87	12.10	3.64	51.80	0.75
Sope Creek	Urban	3/18/2004	19.39	0.217	7.63	7.66	3	49.10	0.70
Plant McDonough	Coal	3/5/2004	15.84	0.296	7.57	6.88	2.62	40.00	1.77
Plant Wansley	Coal	3/3/2004	12.69	1.439	7.99	10.85	10.97	65.10	0.28
Plant Yates	Coal	3/3/2004				6.42	9.17	49.50	1.72
Anneewakee Creek	Wastewater	6/18/2004	25.7	0.085	7.51	3.78	7.14	45.50	1.35
Big Creek	Wastewater	3/29/2004	16.97	0.236	7.96	7.19	5.24	40.00	0.87
Suwanee Creek	Wastewater	6/18/2004	24.85	0.145	7.62	3.27	9.83	36.70	1.50

Table 2.3. Pearson's correlations (*r*) between trace element concentrations in clam tissues across all study sites. Bold type indicates r ≥ 0.50. Significant correlations ($p < 0.05$) for r ≥ 0.17.

	Ni	**Cu**	**Zn**	**As**	**Se**	**Cd**	**Hg**	**Pb**
Ni	1.0000	-	-	-	-	-	-	-
Cu	0.3442	1.0000	-	-	-	-	-	-
Zn	0.4752	**0.7342**	1.0000	-	-	-	-	-
As	0.4680	**0.5216**	**0.5908**	1.0000	-	-	-	-
Se	0.4290	**0.6783**	**0.5856**	**0.7107**	1.0000	-	-	-
Cd	0.2845	**0.5406**	0.4820	0.3349	0.4102	1.0000	-	-
Hg	0.3461	0.0702	-0.0853	0.0300	-0.0427	0.1821	1.0000	-
Pb	0.1223	0.2252	0.3075	0.1628	0.0497	0.2972	-0.1512	1.0000

Table 2.4. Results of the NMS ordination. Correlation of trace element concentrations and environmental variables with NMS axes ($r^2 > 0.30$ reported). Direction of the relationship noted in parentheses.

Variable	**Axis 1**	**Axis 2**
Ni	-	-
Cu	(+)0.35	(-)0.45
Zn	(+)0.97	-
As	-	-
Se	-	-
Cd	(+)0.37	-
Hg	-	(+)0.42
Pb	-	-
% urban	(+)0.39	(-)0.45
% agriculture	(-)0.34	-
% forest	-	(-)0.47
DOC	(+)0.50	-
Conductivity	(+)0.34	-

Figure legend.

Figure 2.1 Map of all fifteen study sites. Latitude and longitude are noted on the x and y axes.

Figure 2.2. Regression of cadmium concentrations in clam tissue versus clam shell length (mm).

Figure 2.3. Mean concentrations of Cd and Ni with 1SE (μg g^{-1} DM) in *Corbicula fluminea* (N=9) from each study site. Hatched bars represent Cd concentrations and black bars represent Ni concentrations.

Figure 2.3. Mean concentration of Cu with 1SE (μg g^{-1} DM) in *Corbicula fluminea* (N=9) from each study site.

Figure 2.5. Mean concentration of Zn with 1SE (μg g^{-1} DM) in *Corbicula fluminea* (N=9) from each study site.

Figure 2.6. Mean concentrations of Se and As with 1SE (μg g^{-1} DM) in *Corbicula fluminea* (N=9) from each study site. Hatched bars represent Se concentrations and black bars represent As concentrations.

Figure 2.7. Mean concentrations of Hg and Pb with 1SE (μg g^{-1} DM) in *Corbicula fluminea* (N=9) from each study site. Hatched bars represent Hg concentrations and black bars represent Pb concentrations.

Figure 2.8. Final NMS ordination plotted with environmental variables correlated with the ordination. Filled squares = forest sites, filled triangles = agricultural sites, filled diamonds = urban sites, open squares = coal sites, and open triangles = wastewater sites. Labeled arrows indicate environmental and land cover variables. The length of the line corresponds to the strength of the correlation with NMS space; correlations range from r^2 = 0.34 to 0.50.

Figure 2.1

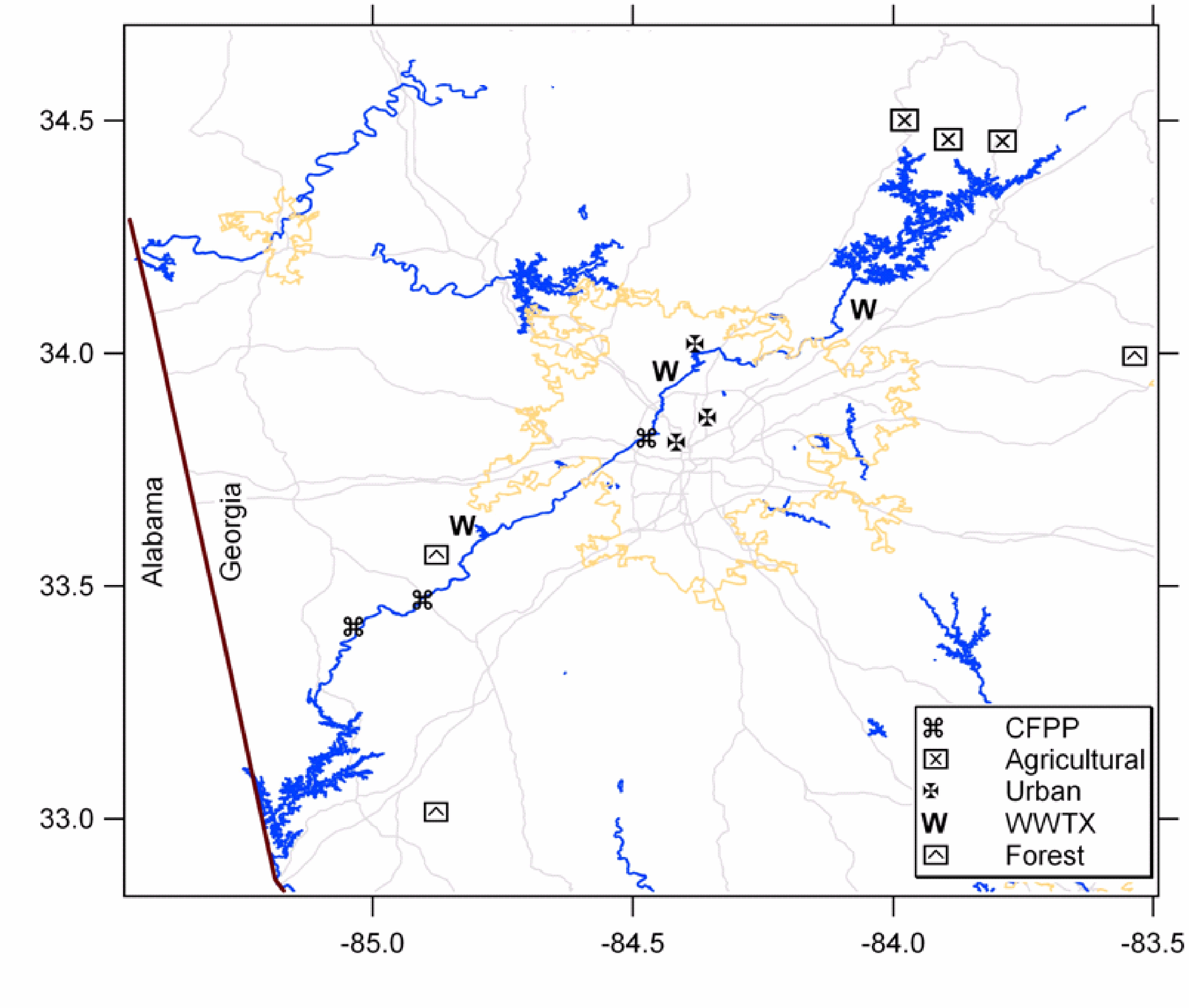

34.5
34.0
33.5
33.0
-85.0
-84.5
-84.0
-83.5
Alabama
Georgia
W
CFPP
Agricultural
Urban
WWTX
Forest

Figure 2.2

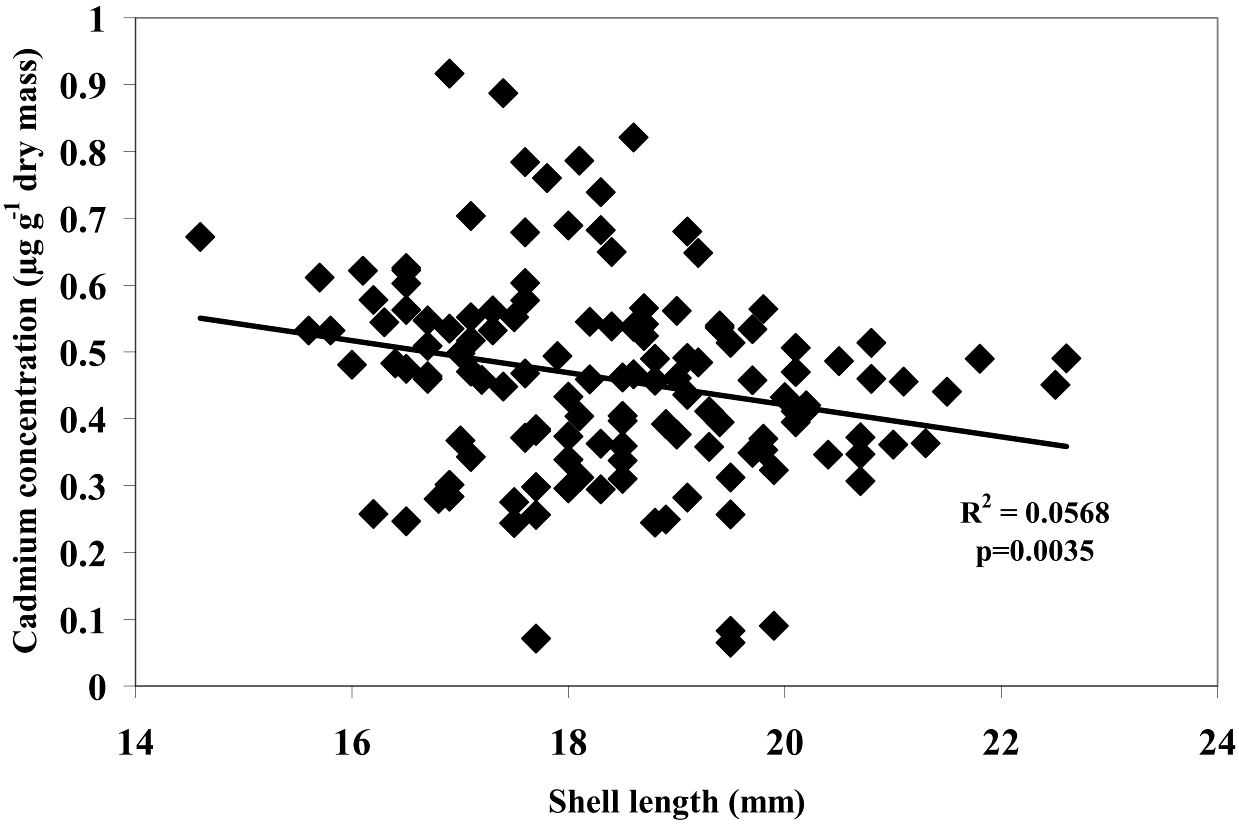

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Cadmium concentration (µg g-1 dry mass)
14
16
18
20
22
24
Shell length (mm)
R2 = 0.0568
p=0.0035

Figure 2.3

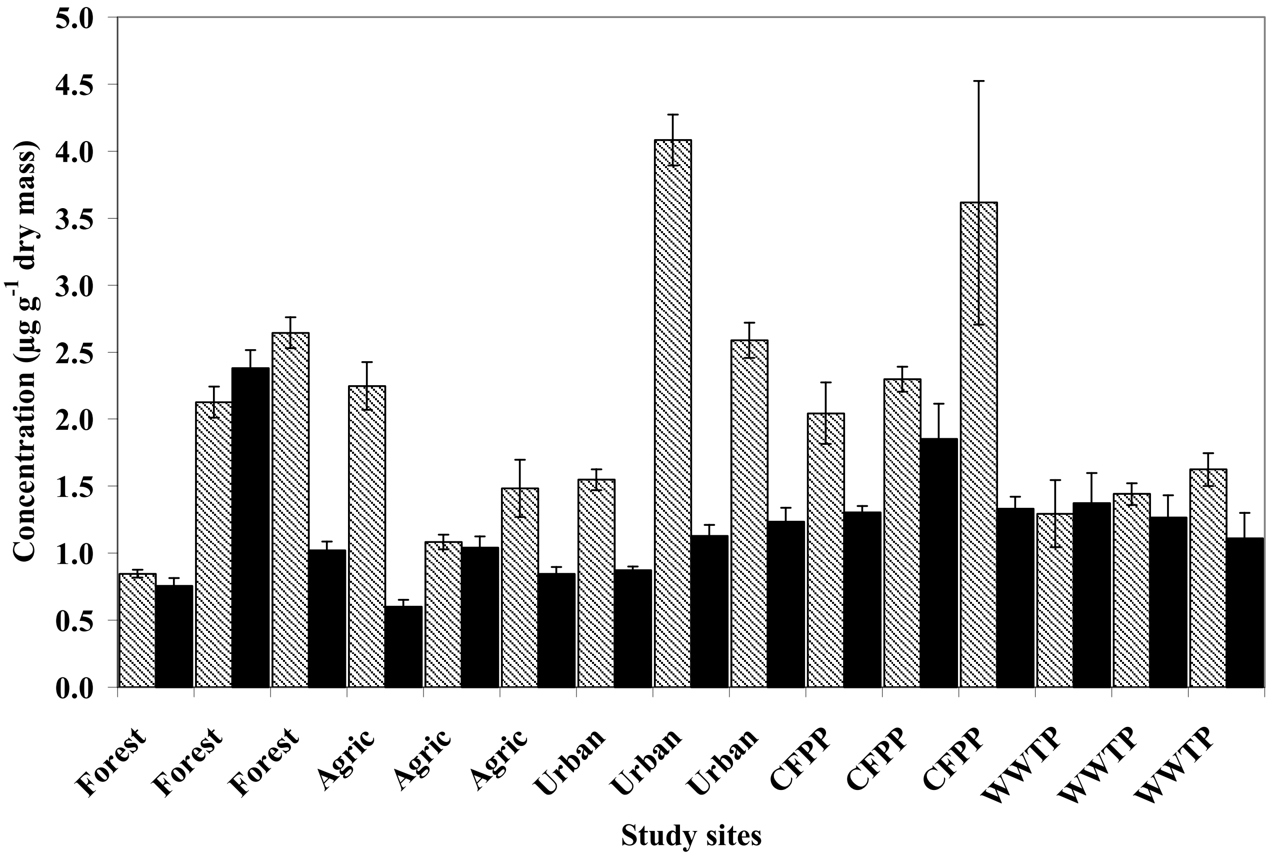

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Concentration (µg g-1 dry mass)
Forest
Forest
Forest
Agric
Agric
Agric
Urban
Urban
Urban
CFPP
CFPP
CFPP
WWTP
WWTP
WWTP
Study sites

Figure 2.4

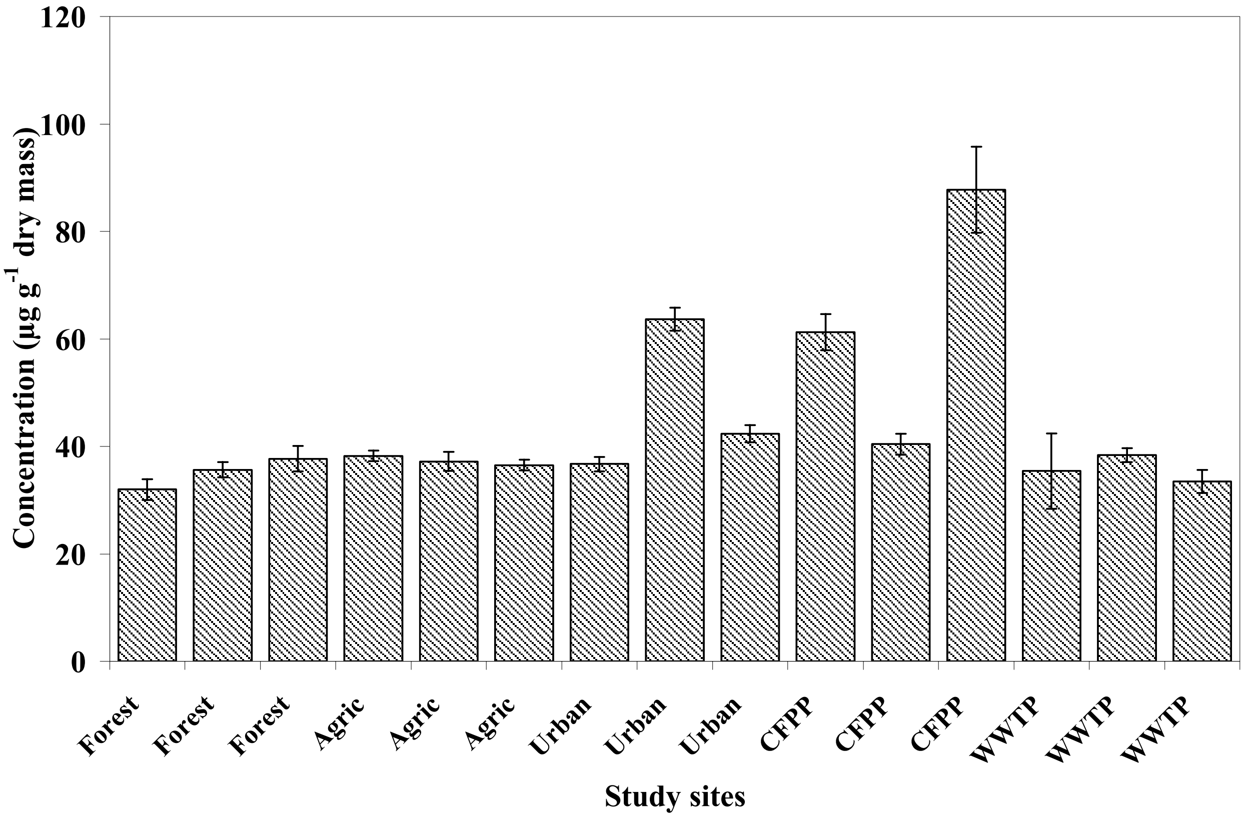

120
100
80
60
40
20
0
Concentration (µg g-1 dry mass)
Forest
Forest
Forest
Agric
Agric
Agric
Urban
Urban
Urban
CFPP
CFPP
CFPP
WWTP
WWTP
WWTP
Study sites

Figure 2.5

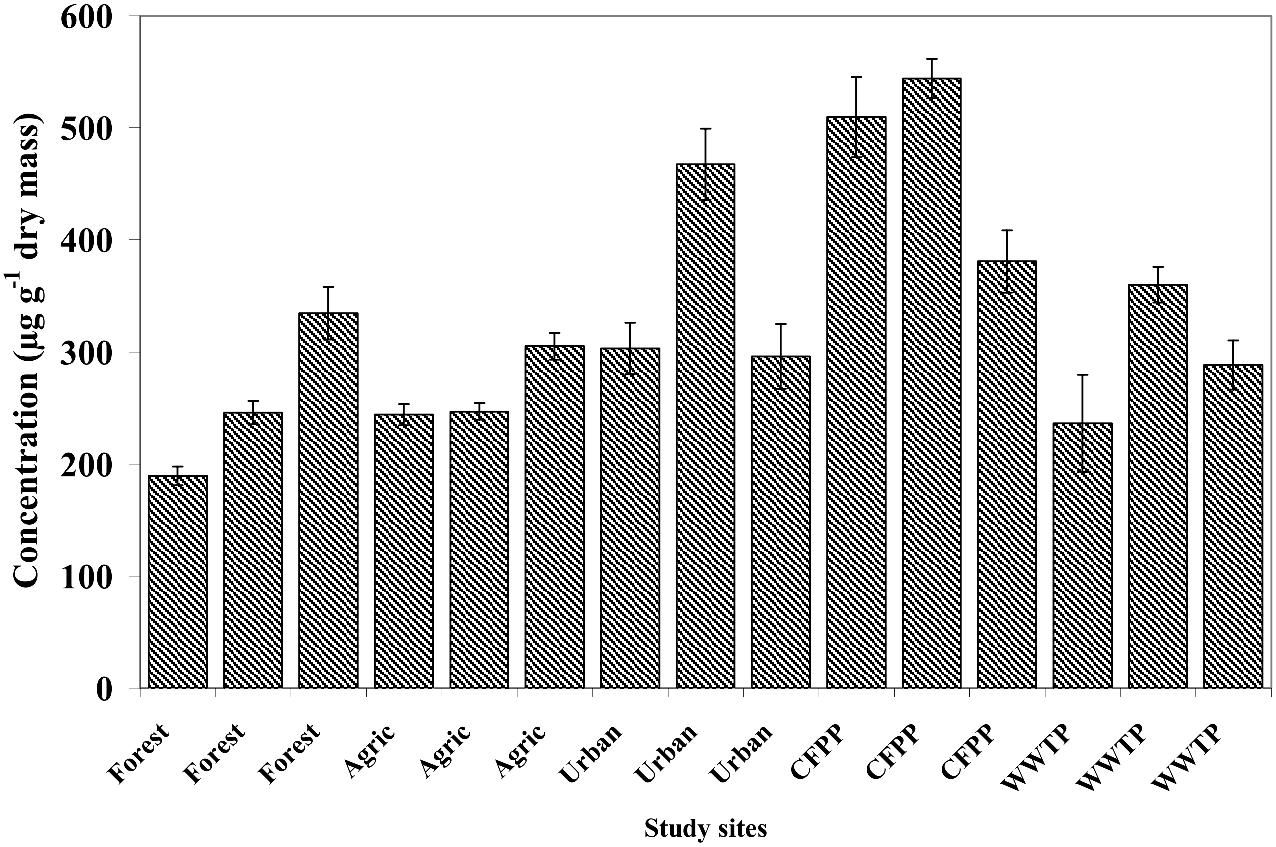

600
500
400
300
200
100
0
Concentration (µg g-1 dry mass)
Forest
Forest
Forest
Agric
Agric
Agric
Urban
Urban
Urban
CFPP
CFPP
CFPP
WWTP
WWTP
WWTP
Study sites

Figure 2.6

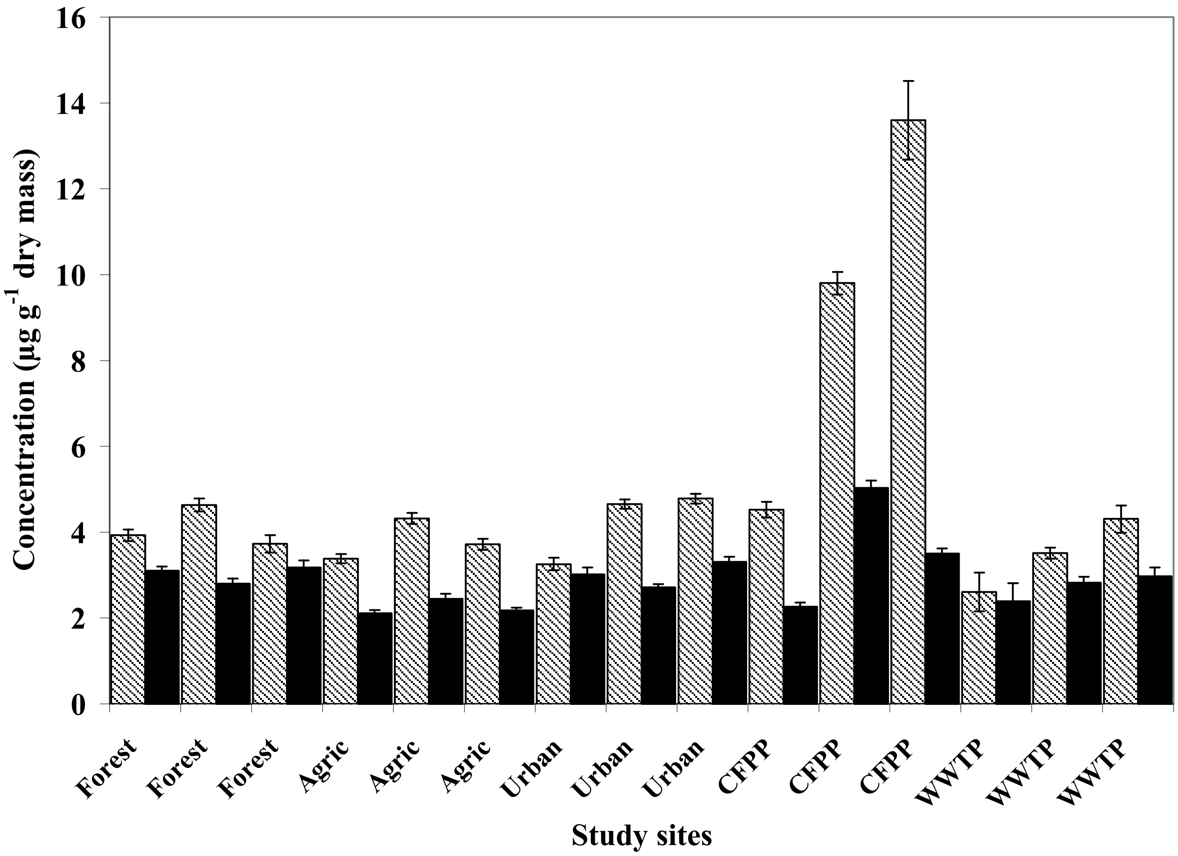

16
14
12
10
8
6
4
2
0
Concentration (µg g-1 dry mass)
Forest
Forest
Forest
Agric
Agric
Agric
Urban
Urban
Urban
CFPP
CFPP
CFPP
WWTP
WWTP
WWTP
Study sites

Figure 2.7

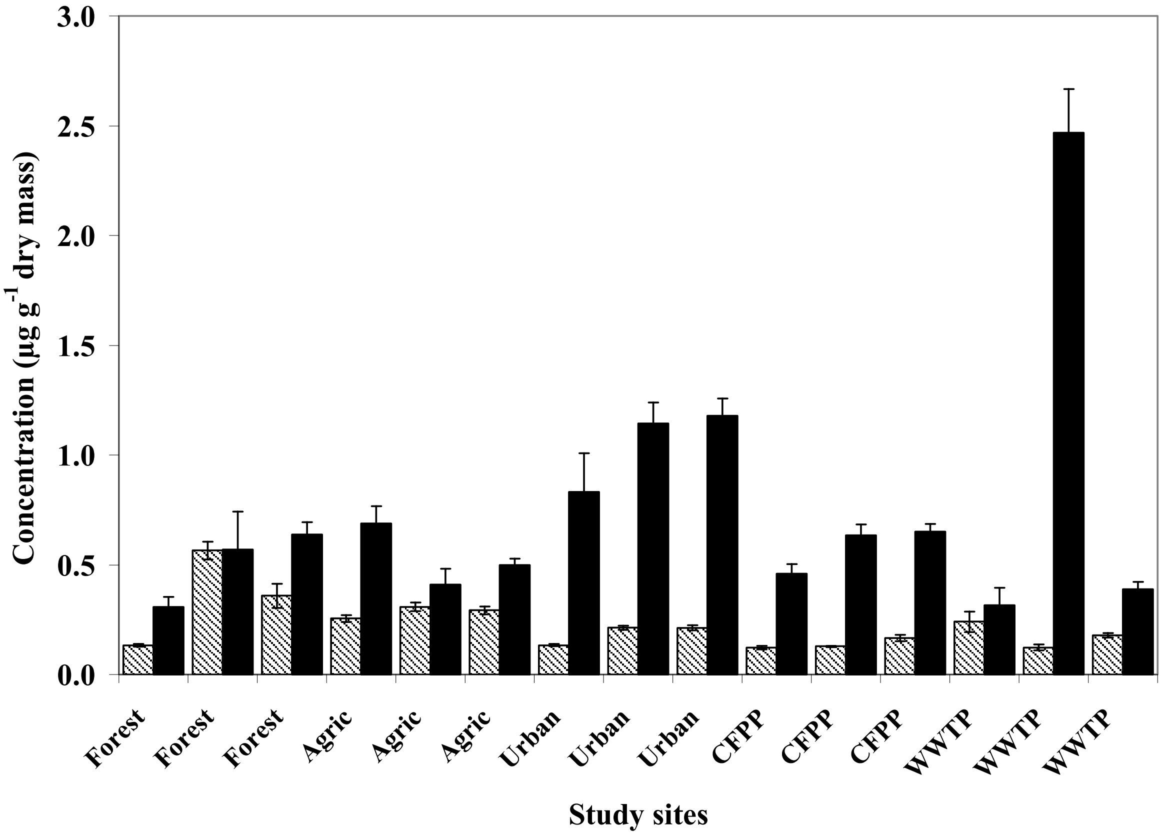
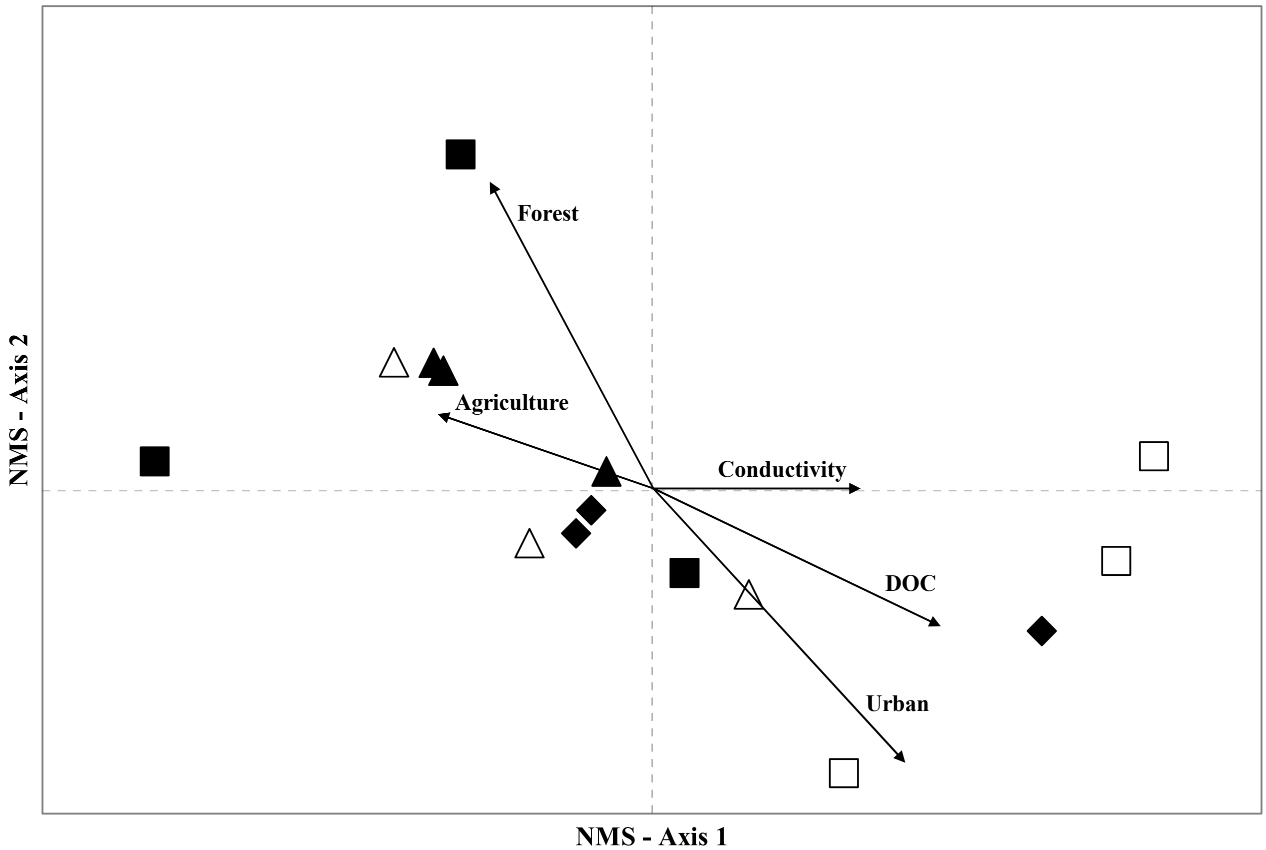

3.0
2.5
2.0
1.5
1.0
0.5
0.0
Concentration (µg g-1 dry mass)
Forest
Forest
Forest
Agric
Agric
Agric
Urban
Urban
Urban
CFPP
CFPP
CFPP
WWTP
WWTP
WWTP
Study sites

Figure 2.8


Forest
Agriculture
Conductivity
DOC
Urban
NMS - Axis 2
NMS - Axis 1

CHAPTER 3

ACCUMULATION OF TRACE ELEMENTS AND GROWTH RESPONSES IN *CORBICULA FLUMINEA* ALONG A GRADIENT OF CONTAMINATION[1]

---

[1]Peltier, Gretchen Loeffler, Meredith S. Wright, William A. Hopkins, and Judy L. Meyer. To be submitted to *Environmental Science and Technology*.

Abstract

Higher concentrations of arsenic, selenium, and cadmium have been observed in *Corbicula fluminea* downstream of coal-fired power plant discharges. To explore the temporal pattern and consequences of trace element accumulation in *Corbicula fluminea*, we transplanted clams from a reference stream to a stream receiving coal-fired power plant discharge. We assessed trace element accumulation and glutathione concentration in clam tissue, shell growth, and condition index at five sites along a contamination gradient over 3 months. Clams at the most upstream and contaminated site (A) had the highest growth rate, highest condition index, and highest concentrations of arsenic (7.85 ± 0.25 μg/g DM (dry mass), selenium (17.75 ± 0.80 μg/g DM), and cadmium (7.28 ± 0.34 μg/g DM). Glutathione concentrations in clams were significantly higher at Site A than the reference site. Ni, Cu, As, Se, and Cd concentrations were highly correlated ($r > 0.60$) in water and tissue. Hg concentrations declined in clams transplanted into the selenium-rich environment of Site A compared to other less impacted, downstream sites. Differences in growth rate and condition index were attributed to differences in thermal conditions among transplant locations rather than trace element concentrations. Thermal benefits diminished the negative effects of trace element contamination, which was detectable with the biomarker glutathione.

Introduction

Half of electricity in the United States is produced by coal combustion (Rowe et al. 2002). Between 1993 and 2004, consumption of coal for energy generation increased from 847 million tons to over 1 billion tons (USDOE 2005). In 1998, approximately 57 million tons of fly ash was produced, which comprises 60% of the coal combustion waste stream. Coal combustion waste contains metals and metalloids such as nickel, copper, zinc, arsenic, selenium, cadmium, mercury, and lead; it is disposed into landfills, aquatic basins, and minefills (Rowe et al. 2002). In this paper we use the term trace elements to include both metals (e.g. zinc, copper, nickel, cadmium) and metalloids (e.g. arsenic and selenium).

A third of coal combustion wastes are disposed into aquatic basins and are 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 1993b, 1997, 2002, Rowe et al. 2002). For example, 19 of 20 fish species in Belews Lake went extinct or were extirpated after exposure to coal-fired power plant (CFPP) discharges containing elevated concentrations of selenium (Lemly 2002). Extirpation of largemouth bass (*Micropterus salmoides)* and significant reductions in bluegill (*Lepomis macrochirus)* populations in Hyco Reservoir were caused by elevated selenium concentrations in coal effluent (Crutchfield 2000). Effects of coal ash on several aquatic species has been investigated in an ash basin and adjacent swamp at the Savannah River (Hopkins et al. 1998, Hopkins et al. 2000, Hopkins et al. 2004b). After four months exposure to coal ash sediments, lake chubsuckers (*Erimyzon sucetta*) exhibited decreased growth, high mortality, and severe fin erosion (Hopkins et al. 2000, Hopkins et al. 2004b). Decreased standard metabolic rate in the crayfish, *Procambarus acutus,* was observed after 27 days of exposure to coal ash sediments (Rowe et al. 2001).

Coal combustion waste is also released into lotic systems; however, little is known about its impacts in rivers and streams (Rowe et al. 2002). In Stingy Run and Little Scary Creek, two streams receiving coal ash effluent, bluegill (*Lepomis macrochirus*) had significantly higher liver concentrations of copper, arsenic, selenium compared to Ohio River reference sites, but growth responses did not significantly differ among sites (Lohner et al. 2001a). Dilution probably plays an important role in reducing the impact of contaminated wastes in lotic environments, but aquatic organisms living immediately downstream of the discharges may be negatively affected. Hotspots of invertebrates with high body burdens of trace elements may exist, resulting in opportunities for transfer of trace elements to higher trophic levels. Elevated trace element concentrations in the tissues of *Corbicula fluminea* have been observed downstream of discharges from CFPPs in an urban river (Chapter 2).

Solely analyzing concentrations of trace elements in *Corbicula fluminea* does not provide insight into physiological responses (e.g. growth and stress), which may or may not occur as a result of elevated tissue concentrations. Positive correlations between concentrations of metals and the biomarker metallothionein (a metal-binding protein) were observed in the tissue of *Corbicula fluminea* as well as reductions in growth downstream of a zinc ore facility (Baudrimont et al. 1999). In contrast, growth of *Corbicula fluminea* was positively correlated with nitrate concentrations and abundance of hydropyschid caddisflies, but unrelated to acid mine drainage inputs containing a mixture of trace elements (Soucek et al. 2001). Glutathione is an antioxidant that may protect against metal toxicity associated with oxidative stress (Stohs and Bagchi 1995, Ciocan and Rotchell 2004). Glutathione 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). In contrast, glutathione 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 (*Mytilus galloprovincialis*, *Scapharca inaequivalvis, and Tapes philippinarum*) living in metal polluted sediments (Irato et al. 2003). Evaluating physiological responses (e.g. biomarkers and growth) in combination with contaminant concentrations enables one to determine if accumulation of contaminants in tissues is negatively affecting an organism.

To explore the temporal pattern and consequences of trace element accumulation in *Corbicula fluminea*, we conducted a transplant study in a lotic system receiving input from only one point source, discharge from the ash pond of a CFPP. The objectives of the study were to quantify the responses of *Corbicula fluminea* transplanted from a reference stream to stream sites along a gradient of coal-ash contamination by analyzing: 1) accumulation of trace elements in whole body tissues of the clams; 2) shell growth rates; 3) glutathione concentrations in clam digestive glands as a measure of stress at the most contaminated site. We predicted that trace element concentrations in clams would be highest at the site closest to discharges from the CFPP and that these clams would have the lowest growth rates and highest concentrations of glutathione.

Methods

Site description

The five study sites were located on the U.S. Department of Energy's Savannah River Site (SRS) near Aiken, SC (Figure 1). The contaminated study stream, Beaver Dam Creek, is located near a CFPP that has been in operation since 1952. 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. 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, B, C, and D. Site A was immediately downstream of the outfall from the lentic habitat; Site B was approximately 400 m downstream from A; Site C was another 1000 m downstream; and Site D was approximately 3.2 km downstream from Site A. 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 5,085 ha (McArthur and Tuckfield 2000).

Experimental design

On the day before the transplant experiment began, approximately 400 clams (*Corbicula fluminea*) were collected from the reference site, Meyers Branch. Clams were kept in aerated site water, returned to the lab, 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 glutathione concentrations were determined using 16 clams from this collection. At each site, four plastic boxes (31x18x11 cm) with holes drilled along the sides were secured with aluminum poles. After adding sediment from the site and 20 clams from the reference site, cages were covered on the top with netting (1 cm mesh) to prevent loss of clams and sediment. 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 19-20, 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. Clams collection occurred 28, 56, and 84 days after transplantation, and clam mortality was less than 1%. The length of the study was chosen to allow for detection of differences in shell growth of clams. On each date, we collected 2 clams from each cage for a total of 8 clams from each site for trace element analysis. At Site A and Meyers Branch, we also collected 2 clams from each cage for a total of 8 clams at each date for glutathione analysis. All clams were weighed and measured again (length, width, and depth) prior to dissection. Using length, width, and depth measurements to calculate volume, we calculated condition index (g dry tissue mass/cm^3 volume) in transplanted clams among the study sites on all dates. 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 most contaminated site.

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 hour at 500°F) 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 (1cm, 500 mm, 250 mm, 2 mm) 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, 84 sampling dates at each study site were ashed at 460 °C for 8 hours to determine their organic matter content.

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 glutathione 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 minutes). A 25 µl subsample of the supernatant was added to 700 µl of sodium phosphate buffer (which contained β-NAPH), 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 minutes. 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 seconds for a total of 120 seconds. Glutathione concentrations were expressed as nmol/g wet mass.

Trace element sampling and analysis

Unfiltered water was collected in acid-washed polyethylene bottles for total trace element concentrations. Separate water samples were filtered in the field using GHP Acrodisc GF 25 mm syringe filters with GF/0.45 µm GHP membranes (Pall Life Sciences, East Hills, NY, USA) for dissolved trace element analyses. Both filtered and unfiltered samples were 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 whirlpak bags, frozen at –70°C, and freeze-dried. Clams being analyzed for trace elements were held in aerated site water for 24 hours. Shell length, width, depth, and weight were measured before freezing clams at –70°C. After thawing, tissues were dissected, frozen at -70°C, 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, 80% microwave power for 10, 10, 15, and 20 minutes, respectively. After digestion with nitric acid, 0.5 ml of trace metal grade hydrogen peroxide was added to the samples and 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. All trace element analyses were performed using an inductively coupled plasma mass spectrometer (ICP-MS) (Perkin Elmer, Norwalk, CT) following previously published methods (Hopkins et al. 2004a). We measured total concentrations of vanadium (V), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), cadmium (Cd), mercury (Hg), and lead (Pb). Concentrations of V, Mn, Fe, Co, Sr will not be discussed in this paper, but all of the trace element concentrations for tissues, sediments, and water are reported in Appendix B. Certified reference materials with similar matrices (Riverwater and Tort 2; 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 – 125%, whereas sediment recoveries ranged from 77 - 124%. Concentrations were expressed as μg/L for water samples and μg/g DM for tissues and sediments.

Statistical analyses

All tissue trace element and glutathione concentrations are reported as means from eight individuals with standard errors. We initially compared tissue concentrations of each trace element in transplanted *Corbicula fluminea* among the five study sites using repeated measures 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, we report and compare mean tissue concentrations of eight clams at each study site rather than cage means. We used repeated measures ANOVA to compare differences in mean trace element concentrations in tissues from resident and transplanted clams from Site A at all times. Repeated measures ANOVA was also used to compare mean glutathione concentrations in clams between Meyers Branch and Site A at 28, 56, and 84 days. Differences in shell growth rate of clams among the five study sites were determined using analysis of covariance (ANCOVA). All analyses used a p-value <0.05 and were run using JMP 5.0.1 or SAS 9.1 (SAS Institute, Cary, NC, USA).

Results

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). Site A had the highest specific conductance, hardness, and temperature, and Meyers Branch had the lowest specific conductance and temperature (Table 3.1). Sediment particle size was dominated by < 2mm size class at all study sites, and sand comprised > 90% of the less than 2 mm size class. Sediment organic matter content ranged from 0.36% at Site A to 2.32% at Site D.

Trace element concentrations

Mean dissolved concentrations of trace elements showed distinct differences among the study sites, but these differences were not evident in the sediments. Dissolved concentrations of Ni, Cu, As, and Se were significantly higher at Site A, and concentrations were lowest at Meyers Branch (Table 3.2). Zn concentrations were significantly lower at Site A, but the mean concentrations at the other four sites were similar (Table 3.2). Concentrations of Cd were higher at Site A than at other sites, and Pb concentrations did not differ among sites.

Concentrations of trace elements in the tissue of transplanted clams differed significantly between Meyers Branch (reference) and Site A (Figure 3.1). Ni, As, Se, and Cd tissue concentrations were significantly higher at Site A than at all other sites (Figure 3.1). Other trace elements (Hg, Pb, Zn, and Cu) exhibited different patterns. Hg concentrations in the tissues of transplanted clams at Site A decreased during the experiment and by day 84 were the lowest measured among the study sites (Figure 3.1). Pb concentrations spiked at Meyers Branch at day 56, but did not differ among the sites on other dates (Figure 3.1). Zn and Cu concentrations were higher at Site A on day 28, but declined over the remainder of the study period (Figures 3.1). Trace element tissue concentrations of transplanted clams at Sites B, C, and D were similar throughout the experiment (Figure 3.1). Ni, Cu, As, Se, and Cd tissue concentrations in transplanted clams were highly correlated with dissolved concentrations ($r > 0.60$, $p < 0.005$). 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 (e.g, arsenic and selenium, Figure 3.2).

To better understand the spatial extent of contamination downstream of Site A, we collected clams, water, and water quality parameters in June 2005 from Site A, Site B, and an intermediate site (A1) approximately 200 m downstream of Site A. Tissue and water concentrations at Sites A and B were similar to 2004 measurements. Tissue concentrations of Ni, Cu, As, Se, and Cd were significantly higher (ANOVA, $p<0.05$) in clams from Site A compared with clams from Sites A1 and B. Tissue concentrations in clams from Sites A1 and B (reported in Appendix B) did not differ significantly for any trace element.

Trace element concentrations in resident clams at Site A were significantly different than transplanted clams (Figure 3.3). Ni, Cu, Zn, As, Se, and Cd concentrations were higher in resident clams (Figure 3.3). Hg concentrations in resident clams were lower than transplanted clams at Site A (Figure 3.3). However, Hg declined in transplanted clams until it reached a concentration more similar to that in resident clams. Despite differences in total concentrations, temporal trends in trace element tissue concentrations of As, Se, and Cd in resident and transplanted clams were similar (Figures 3.3). 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 (Figure 3.3).

Growth and stress responses

Clams from Site A had the highest growth rate (Figure 3.4) and condition index (Figure 3.5), which is contrary to our original hypotheses. Clam growth rate was significantly higher at Site B than at the reference site, but significantly less than at Site A. Log [growth rate] increased with mean temperature across sites ($r^2 = 0.68$, $p = 0.0001$) (Figure 3.6).

Glutathione concentrations in clams were significantly higher at Site A than Meyers Branch on all sampling dates (Figure 3.7). Higher concentrations of glutathione can be interpreted as induction of cellular defense mechanisms in response to contaminant exposure (Irato et al. 2003).

Discussion

Trace element concentrations

Trace element concentrations significantly differed between Site A and Meyers Branch. However, tissue and water concentrations of Ni, Cu, Zn, As, Cd, and Pb at the three downstream sites in Beaver Dam Creek (Sites B, C, and D) were very similar to concentrations at Meyers Branch (Figure 3.1), which suggests that rapid dilution of contaminants is occurring downstream from the power plant. The trace element concentrations in water and clam tissue from summer 2005 (Sites A, A1, B) provide additional evidence that rapid contaminant dilution occurs below Site A.

Tissue concentrations of all trace elements (except Hg and Pb) reached maximum levels by day 28 (Figure 3.1), suggesting rapid accumulation occurred after introduction into the contaminated environment. Tissue concentrations of Cd in resident and transplanted clams decreased after day 28, and it is unclear if concentrations would continue to decrease after day 84 or stabilize as As and Se concentrations did.

Cu and Cd concentrations peaked at day 28 and declined for the remaining 56 days in both transplanted and resident clams at Site A (Figure 3.3). The spike in concentration may be a result of increased precipitation (Figure 3.8) or discharges of trace elements that occurred before day 28 of the experiment. 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. Correlations between tissue and dissolved concentrations were driven by high concentrations at the contaminated site and were not significant when the site was removed from the analysis (Figure 3.2).

The transplant experiment demonstrated the antagonistic relationship between selenium and mercury that has been observed in other studies (e.g., Chen et al. 2001, Hamilton 2004). 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 because when water concentrations exceed 3 μg/L, 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 sites between Se and Hg, formation of Hg-Se complexes, or increased activity of glutathione peroxidase (Belzile et al. 2006). Clams from the reference site had high tissue concentrations of total Hg. When these clams were transplanted into the selenium-rich environment of Site A, Hg concentrations declined while selenium concentrations increased (Figure 3.1). Hg concentration in clams transplanted to Sites B-D also declined, but not to the extent seen at Site A (Figure 3.1).

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, stress associated with living in a contaminated environment (Site A) was confounded by warmer temperatures at this site. Elevated temperatures stimulate growth rates in bivalves (McMahon 1991, Cataldo and Boltovskoy 1998). In one-year-old clams, a 17° C decrease in water temperature was associated with a 7-fold decrease in growth rate (Cataldo and Boltovskoy 1998). Clams at Site A may also have more plentiful food resources than the other study sites. *Corbicula fluminea* downstream of a swamp had higher condition indices than clams from upstream environments likely because of higher seston concentrations (Kesler 2004).

Glutathione concentrations can signal that oxidative stress is occurring in response to metal or organic contaminant exposures (Stohs and Bagchi 1995). Differences in glutathione concentration between Site A and Meyers Branch imply that defense mechanisms were induced in clams from Site A (Irato et al. 2003) in response to oxidative stress associated with metal exposure. Many studies show that glutathione concentrations change in response to contaminant exposure (e.g., Yan et al. 1997, Canesi et al. 1999, Ringwood et al. 1999). In juvenile oysters (*Crassostrea virginica*), glutathione concentrations were reduced after in-situ exposure to metals and PAHs (Ringwood et al. 1999). However, glutathione concentrations in the digestive gland increased in the green mussel (*Perna viridis*) after exposure to Hg and Pb (Yan et al. 1997). Concentrations also increased in three bivalves (*Mytilus galloprovincialis*, *Scapharca inaequivalvis, and Tapes philippinarum*) living in metal polluted sediments (Irato et al. 2003).

Clams in this study had lower glutathione concentrations than bivalves from other metal-contaminated environments with similar or lower exposures to Ni, Cu, Zn, and Cd. Concentrations of glutathione in clams from both Site A and Meyers Branch were more than an order of magnitude lower than concentrations (> 600 nmol/g wet mass) in mussels (*Mytilus galloprovincialis*) exposed to lower concentrations of Cu and MeHg than in this study (Canesi et al. 1999). Glutathione concentrations were about 20 times lower than observed in three bivalve species (500 – 1500 nnol/g wet mass) exposed to similar concentrations of Ni, Cu, Zn, and Cd contamination in the field (Irato et al. 2003). In the laboratory, glutathione concentrations in *Corbicula fluminea* are consistently orders of magnitude lower than in fish livers (D. Conners, personal communication). The differences in glutathione concentration described above may be related to interspecies differences among bivalves rather than differences in exposure. The data suggest that detoxification mechanisms were induced in clams from Site A. Trace element concentrations may be high enough to initiate detoxification processes, but not enough to reduce growth or reproduction. Other variables such as warmer water temperatures and increased food resource availability at the contaminated site appear to diminish the negative effects of elevated trace element concentrations.

Contamination at other coal-fired power plants

Trace element concentrations in the tissues of clams from Site A at the Savannah River Site are slightly higher than concentrations found in clams below CFPP discharges in the

Chattahoochee River in Atlanta, GA (Table 3.3). 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 the Chattahoochee sites (Table 3.3). Dissolved concentrations of As and Se at Site A were 18.31 and 2.76 µg/L, respectively compared to ranges of 0.36 – 1.50 and 0.41 – 3.53 µg/L at two of the Chattahoochee River sites (concentrations at the third site are outliers, Appendix A). In the Chattahoochee River, As and Se concentrations in clam tissues are similar to or somewhat less than what would be predicted from dissolved concentrations based on the regression from the Savannah River Site (Figure 3.2.A and B). 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 (3.07 and 1.41 µg/L, respectively; Lesley and Froelich 2003). This suggests that elevated concentrations of trace elements extend far downstream from the discharges despite dilution in this river, which has much higher discharge than the streams at the Savannah River Site. 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 vs. 1730 MW). Trace element accumulation in aquatic organisms downstream of CFPP discharges is a function of several factors including energy generation capacity, river discharge, and proximity of other CFPP discharges in a river.

Conclusions

Accumulation of Ni, As, Se, and Cd was significantly higher in clams transplanted to a site immediately downstream of the discharge from a CFPP than in clams transplanted further downstream or in clams at a reference site. Growth response and condition index in *Corbicula fluminea* were insensitive to the effects of elevated trace element concentrations. However, glutathione concentration was significantly higher at the contaminated site than the reference site. Hg concentrations declined in clams transplanted into the selenium-rich environment of Site A compared to other less impacted, downstream sites. Based on the accumulation of trace elements in resident and transplanted clams, the potential exists for trace element accumulation in other members of the stream food web.

Acknowledgements

We would like to 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 provided by Sarah DuRant, Davis Harrelson, and John Peterson. Special thanks to Marsha Black and Deanna Conners for field equipment and guidance in cage design. Portion of funding through Graduate Research Fellowship given to G.L.P. under Financial Assistance Award DE-FC09-96SR18-546 between the University of Georgia and the U.S. Department of Energy.

Literature Cited

Anderson, M. E. 1985. Determination of glutathione and glutathione disulfide in biological samples. Methods in Enzymology **113**:548-555.

Baudrimont, M., S. Andres, J. Metivaud, Y. Lapaquellerie, F. Ribeyre, N. Maillet, C. Latouche, and A. Boudou. 1999. Field transplantation of the freshwater bivalve Corbicula fluminea along a polymetallic contamination gradient (river Lot, France): II. Metallothionein response to metal exposure. Environmental Toxicology and Chemistry **18**:2472-2477.

Belzile, N., Y. W. Chen, J. M. Gunn, J. Tong, Y. Alarie, T. Delonchamp, and C. Y. Lang. 2006. The effect of selenium on mercury assimilation by freshwater organisms. Canadian Journal of Fisheries and Aquatic Sciences **63**:1-10.

Canesi, L., A. Viarengo, C. Leonzio, M. Filippelli, and G. Gallo. 1999. Heavy metals and glutathione metabolism in mussel tissues. Aquatic Toxicology **46**:67-76.

Cataldo, D., and D. Boltovskoy. 1998. Population dynamics of Corbicula fluminea (Bivalvia) in the Parana River Delta (Argentina). Hydrobiologia **380**:153-163.

Chen, Y. W., N. Belzile, and J. M. Gunn. 2001. Antagonistic effect of selenium on mercury assimilation by fish populations near Sudbury metal smelters? Limnology and Oceanography **46**:1814-1818.

Cherry, D. S., R. K. Guthrie, E. M. Davis, and R. S. Harvey. 1984. Coal ash basin effects (particulates, metals, acidic pH) upon aquatic biota - an 8-year evaluation. Water Resources Bulletin **20**:535-544.

Ciocan, C. M., and J. M. Rotchell. 2004. Cadmium induction of metallothionein isoforms in juvenile and adult mussel (Mytilus edulis). Environmental Science & Technology **38**:1073-1078.

Couillard, Y., P. G. C. Campbell, A. Tessier, J. Pellerinmassicotte, and J. C. Auclair. 1995. Field transplantation of a fresh-water bivalve, Pyganodon grandis, across a metal contamination gradient .1. Temporal changes in metallothionein and metal (Cd, Cu, and Zn) concentrations in soft-tissues. Canadian Journal of Fisheries and Aquatic Sciences **52**:690-702.

Crutchfield, J. U. 2000. Recovery of a power plant cooling reservoir ecosystem from selenium bioaccumulation. Environmental Science & Policy **3**:145-163.

Hamilton, S. J. 2004. Review of selenium toxicity in the aquatic food chain. Science of the Total Environment **326**:1-31.

Hopkins, W. A., M. T. Mendonca, C. L. Rowe, and J. D. Congdon. 1998. Elevated trace element concentrations in southern toads, Bufo terrestris, exposed to coal combustion waste. Archives of Environmental Contamination and Toxicology **35**:325-329.

Hopkins, W. A., J. W. Snodgrass, J. H. Roe, B. P. Jackson, J. C. Gariboldi, and J. D. Congdon. 2000. Detrimental effects associated with trace element uptake in lake chubsuckers (Erimyzon sucetta) exposed to polluted sediments. Archives of Environmental Contamination and Toxicology **39**:193-199.

Hopkins, W. A., B. P. Staub, J. A. Baionno, B. P. Jackson, J. H. Roe, and N. B. Ford. 2004a. Trophic and maternal transfer of selenium in brown house snakes (Lamprophis fuliginosus). Ecotoxicology and Environmental Safety **58**:285-293.

Hopkins, W. A., B. P. Staub, J. W. Snodgrass, B. E. Taylor, A. E. DeBiase, J. H. Roe, B. P. Jackson, and J. D. Congdon. 2004b. Responses of benthic fish exposed to contaminants in outdoor microcosms - examining the ecological relevance of previous laboratory toxicity tests. Aquatic Toxicology **68**:1-12.

Irato, P., G. Santovito, A. Cassini, E. Piccinni, and V. Albergoni. 2003. Metal accumulation and binding protein induction in Mytilus galloprovincialis, Scapharca inaequivalvis, and Tapes philippinarum from the lagoon of Venice. Archives of Environmental Contamination and Toxicology **44**:476-484.

Kesler, D. H. 2004. Influence of a lentic area on condition indices of Corbicula fluminea in the Wolf River, Tennessee. Journal of Freshwater Ecology **19**:445-453.

Lemly, A. D. 1993. Teratogenic effects of selenium in natural-populations of fresh-water fish. Ecotoxicology and Environmental Safety **26**:181-204.

Lemly, A. D. 1997. Ecosystem recovery following selenium contamination in a freshwater reservoir. Ecotoxicology and Environmental Safety **36**:275-281.

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

Lesley, P., and P. N. Froelich. 2003. Arsenic, selenium, and antimony: from coal fired power plants to the Chattahoochee River. *in* K. J. Hatcher, editor. Proceedings of the 2003 Georgia Water Resources Conference. University of Georgia, Athens, GA.

Lohner, T. W., R. J. Reash, V. E. Willet, and L. A. Rose. 2001. Assessment of tolerant sunfish populations (Lepomis sp.) inhabiting selenium-laden coal ash effluents - 1. Hematological and population level assessment. Ecotoxicology and Environmental Safety **50**:203-216.

McArthur, J. V., and R. C. Tuckfield. 2000. Spatial patterns in antibiotic resistance among stream bacteria: Effects of industrial pollution. Applied and Environmental Microbiology **66**:3722-3726.

McMahon, R. F. 1991. Mollusca: Bivalvia. *in* J. H. Thorp and A. P. Covich, editors. Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego.

Ringwood, A. H., D. E. Conners, C. J. Keppler, and A. A. DiNovo. 1999. Biomarker studies with juvenile oysters (Crassostrea virginica) deployed in-situ. Biomarkers **4**:400-414.

Rowe, C. L., W. A. Hopkins, and J. D. Congdon. 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: A review. Environmental Monitoring and Assessment **80**:207-276.

Rowe, C. L., W. A. Hopkins, C. Zehnder, and J. D. Congdon. 2001. Metabolic costs incurred by crayfish (Procambarus acutus) in a trace element-polluted habitat: further evidence of similar responses among diverse taxonomic groups. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **129**:275-283.

Rudd, J. W. M., M. A. Turner, A. Furutani, B. E. Townsend, and A. Swick. 1980. Dynamics of selenium in mercury-contaminated experimental fresh-water ecosystems. Canadian Journal of Fisheries and Aquatic Sciences **37**:848-857.

Sandhu, S. S., G. L. Mills, and K. S. Sajwan. 1993. Leachability of Ni, Cd, Cr, and As from coal ash impoundments of different ages on the Savannah River Site. *in* R. K. Keefer and K. S. Sajwan, editors. Trace elements in coal and coal combustion residues. CRC Press, Boca Raton, FL.

Soucek, D. J., T. S. Schmidt, and D. S. Cherry. 2001. In situ studies with Asian clams (Corbicula fluminea) detect acid mine drainage and nutrient inputs in low-order streams. Canadian Journal of Fisheries and Aquatic Sciences **58**:602-608.

Stohs, S. J., and D. Bagchi. 1995. Oxidative mechanisms in the toxicity of metal-ions. Free Radical Biology and Medicine **18**:321-336.

Turner, M. A., and J. W. M. Rudd. 1983. The English Wabigoon River system .3. Selenium in lake enclosures - Its geochemistry, bioaccumulation, and ability to reduce mercury bioaccumulation. Canadian Journal of Fisheries and Aquatic Sciences **40**:2228-2240.

Yan, T., L. H. Teo, and Y. M. Sin. 1997. Effects of mercury and lead on tissue glutathione of the green mussel, Perna viridis L. Bulletin of Environmental Contamination and Toxicology **58**:845-850.

Table 3.1. Mean water quality parameters (N = 4 dates) at the five study sites over the three 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	0.060 ± 0.002	7.48 ± 0.36	23.63 ± 1.56	23.63 ± 0.99	3.59 ± 0.92
Beaver Dam Creek (A)	30.94 ± 1.64	7.43 ± 0.22	0.293 ± 0.003	7.29 ± 0.48	16.80 ± 1.88	38.00 ± 1.83	1.91 ± 0.22
Beaver Dam Creek (B)	27.76 ± 0.63	7.25 ± 0.16	0.136 ± 0.006	7.02 ± 0.44	26.50 ± 2.87	20.50 ± 0.65	4.56 ± 0.72
Beaver Dam Creek (C)	27.40 ± 0.43	7.27 ± 0.19	0.143 ± 0.008	7.07 ± 0.53	24.03 ± 1.14	20.19 ± 0.94	6.74 ± 1.68
Beaver Dam Creek (D)	27.08 ± 0.47	7.33 ± 0.21	0.141 ± 0.009	7.00 ± 0.38	26.20 ± 3.59	21.19 ± 0.90	7.00 ± 1.60

Table 3.2. Dissolved concentrations of trace elements (mean ± 1SE) at each study site. Concentrations expressed as µg/L. N = 4 sampling dates.

Study site	Ni	Cu	Zn	As	Se	Cd	Pb
Meyers Branch	0.05 ± 0.03	0.35 ± 0.11	26.96 ± 2.12	0.21 ± 0.04	0.27 ± 0.05	BDL	0.07 ± 0.02
Beaver Dam Creek (A)	2.68 ± 0.39	2.53 ± 0.40	18.72 ± 3.79	18.31 ± 3.14	2.76 ± 0.15	0.13 ± 0.03	0.02 ± 0.02
Beaver Dam Creek (B)	0.58 ± 0.12	1.91 ± 0.24	28.49 ± 3.07	1.81 ± 0.44	0.56 ± 0.09	0.03 ± 0.01	0.07 ± 0.02
Beaver Dam Creek (C)	0.58 ± 0.16	1.84 ± 0.23	29.05 ± 3.86	1.69 ± 0.33	0.57 ± 0.07	0.04 ± 0.02	0.12 ± 0.03
Beaver Dam Creek (D)	0.58 ± 0.16	1.52 ± 0.10	25.98 ± 1.17	1.66 ± 0.40	0.51 ± 0.11	0.04 ± 0.02	0.06 ± 0.01

Table 3.3. 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**	**Pb**
SRS-Resident	11.15 ± 1.04	123.66 ± 24.31	277.48 ± 22.34	9.45 ± 0.19	23.81 ± 0.68	12.64 ± 0.65	0.09 ± 0.02	0.19 ± 0.02
SRS-Transplant	6.02 ± 0.58	54.13 ± 2.45	161.99 ± 5.77	7.85 ± 0.25	17.75 ± 0.80	7.28 ± 0.34	0.81 ± 0.11	0.28 ± 0.02
Plant#1	1.30 ± 0.05	61.24 ± 3.34	509.52 ± 35.73	2.26 ± 0.10	4.53 ± 0.19	2.04 ± 0.23	0.12 ± 0.01	0.46 ± 0.04
Plant#2	1.85 ± 0.26	40.39 ± 1.93	543.96 ± 17.65	5.03 ± 0.17	9.80 ± 0.26	2.30 ± 0.09	0.13 ± 0.00	0.63 ± 0.05
Plant#3	1.33 ± 0.09	87.73 ± 7.99	380.75 ± 27.67	3.50 ± 0.12	13.60 ± 0.92	3.61 ± 0.91	0.17 ± 0.01	0.65 ± 0.03

Figure Legend.

Figure 3.1. Tissue concentrations expressed as µg/g dry mass (mean ± 1SE) of Ni, Cu, Zn, As, Se, Cd, Hg, and Pb in transplanted *Corbicula fluminea*. Filled diamonds represent the Meyers Branch (reference) site, filled triangles represent Site A, filled squares represent Site B, open squares represent Site C, and open diamonds represent Site D. Symbols are often larger than standard error bars.

Figure 3.2. A) Mean tissue concentration of arsenic (µg/g dry mass) versus dissolved concentration of arsenic (µg/L) at each site and timepoint. Regression equation: $y = 0.175x + 4.404$, $r^2 = 0.75$, $p < 0.0001$. B) Tissue concentration of selenium (µg/g dry mass) versus dissolved concentration of selenium (µg/L) at each site and timepoint. Regression equation: $y = 4.632x + 5.286$, $r^2 = 0.77$, $p < 0.0001$. Open diamonds represent tissue and dissolved concentrations from the Chattahoochee River (Chapter 2); these points were not included in the regressions.

Figure 3.3. Tissue concentrations expressed as µg/g dry mass (mean ± 1SE) of Ni, Cu, Zn, As, Se, Cd, Hg, and Pb in transplant and resident *Corbicula fluminea* at Site A. Filled diamonds represent transplant clams and open diamonds represent resident clams. Symbols are often larger than standard error bars.

Figure 3.4. Total length (mean ± 1SE) of clams over the course of the experiment at five study sites. Symbols for sites are listed in Figure 3.1. Lines are regressions that were used to determine growth rate. Meyers: $y = 16.973 – 0.001x$; Site A: $y = 16.706 + 0.040x$; Site B: $y = 17.356 + 0.011x$; Site C: $y = 17.200 + 0.005x$; Site D: $y = 17.325 + 0.005x$.

Figure 3.5. Condition index (mean ± 1SE) versus time at five study sites. Symbols for sites are listed in Figure 3.1. Standard error bars are often larger than standard error bars.

Figure 3.6. Plot of $\log_{10}$ [mean growth rate] of *Corbicula fluminea* versus water temperature at all sites. Regression equation: $y = -5.13 + 0.11x$, $r^2 = 0.6832$, $p = 0.0001$. Symbols for sites are listed in Figure 3.1.

Figure 3.7. Glutathione concentration (mean ± 1SE) in transplanted clams from Meyers Branch and Site A. Filled triangles represent Site A and filled diamonds represent Meyers Branch.

Figure 3.8. Daily precipitation record from April – August 2004 at Beaver Dam Creek. Arrows indicate sampling dates.

Figure 3.1

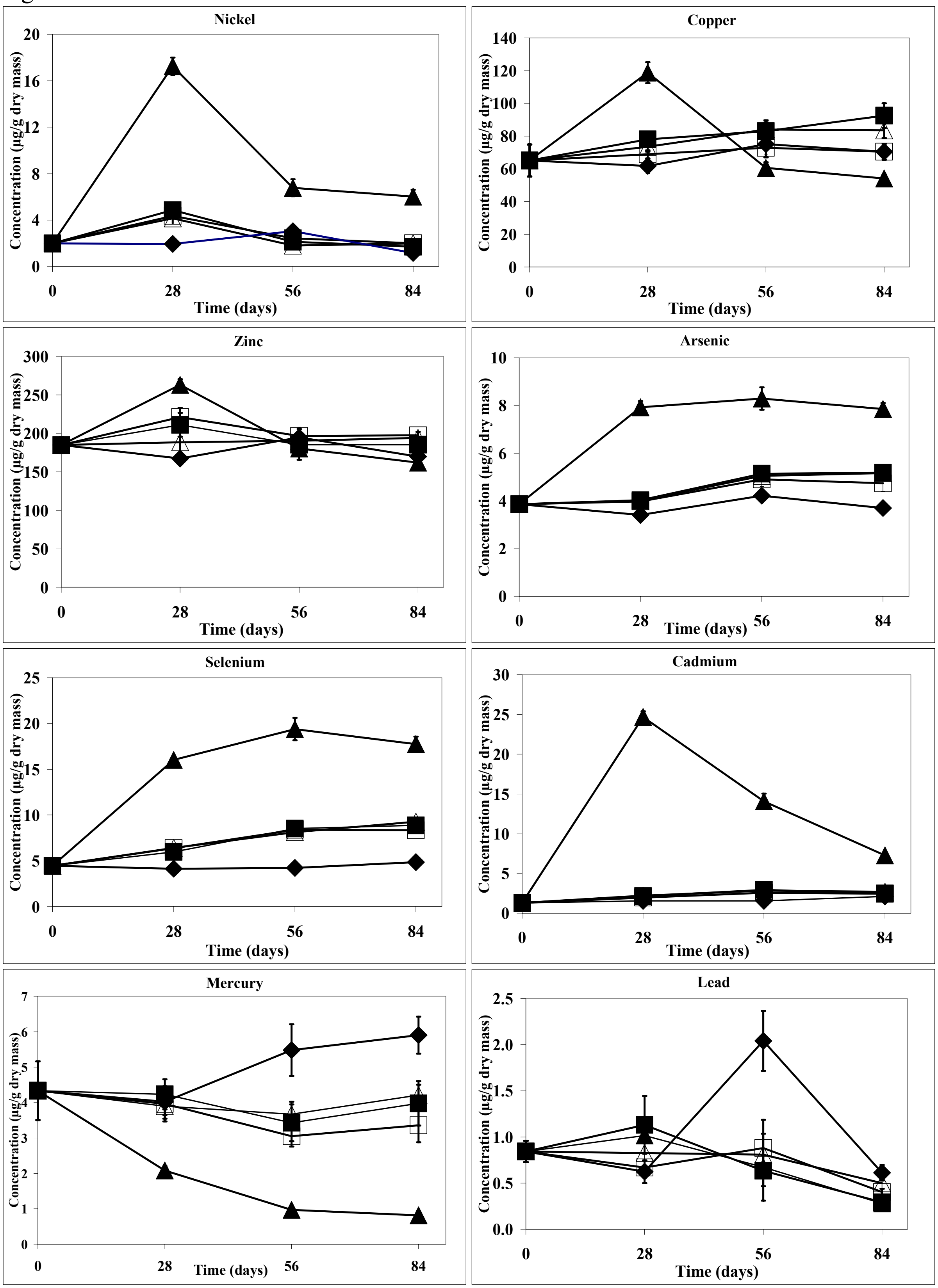

Nickel
Concentration (µg/g dry mass)
0
4
8
12
16
20
0
28
56
84
Time (days)
Copper
Concentration (µg/g dry mass)
0
20
40
60
80
100
120
140
0
28
56
84
Time (days)
Zinc
Concentration (µg/g dry mass)
0
50
100
150
200
250
300
0
28
56
84
Time (days)
Arsenic
Concentration (µg/g dry mass)
0
2
4
6
8
10
0
28
56
84
Time (days)
Selenium
Concentration (µg/g dry mass)
0
5
10
15
20
25
0
28
56
84
Time (days)
Cadmium
Concentration (µg/g dry mass)
0
5
10
15
20
25
30
0
28
56
84
Time (days)
Mercury
Concentration (µg/g dry mass)
0
1
2
3
4
5
6
7
0
28
56
84
Time (days)
Lead
Concentration (µg/g dry mass)
0.0
0.5
1.0
1.5
2.0
2.5
0
28
56
84
Time (days)

Figure 3.2.

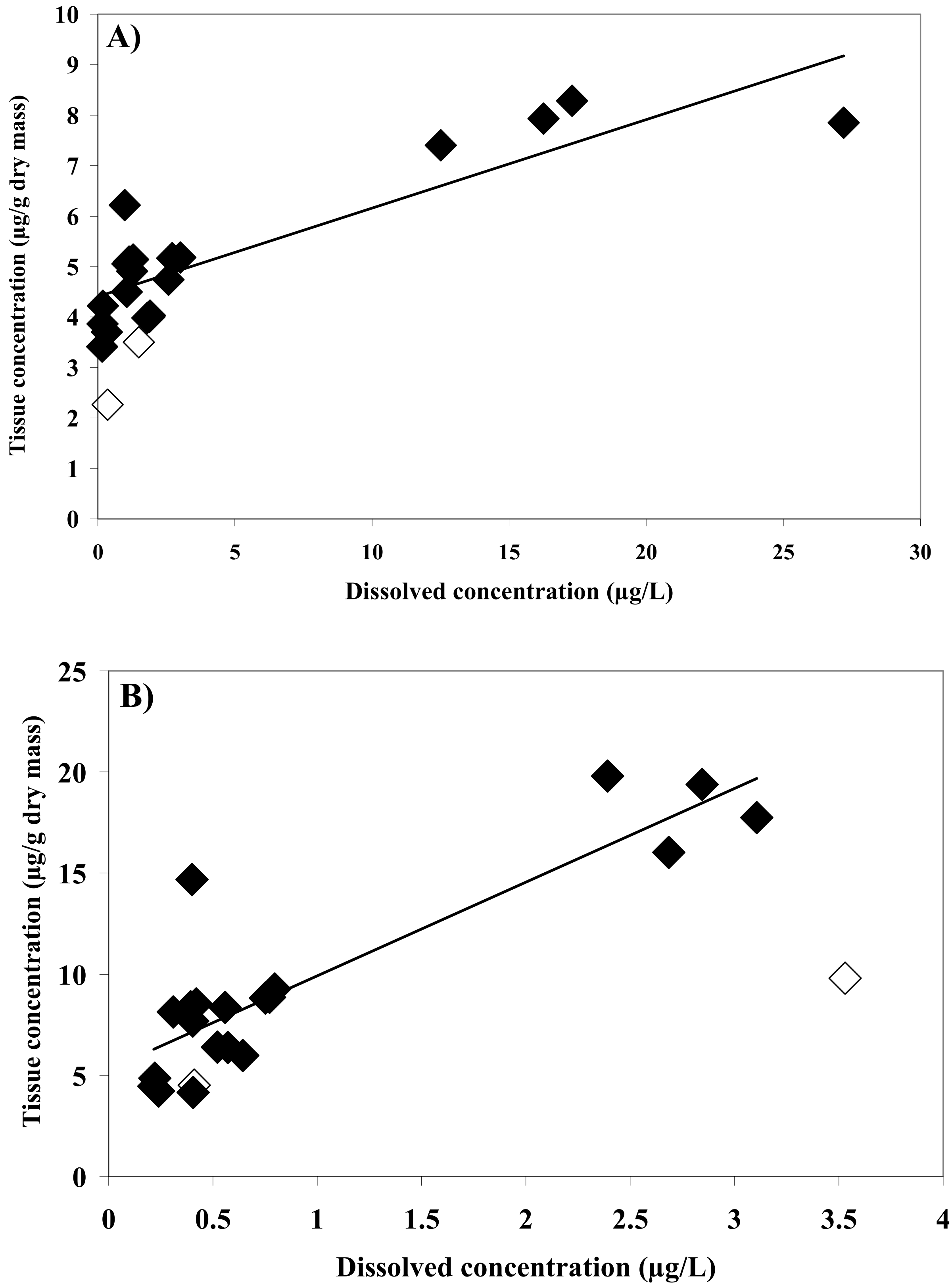

A)
Tissue concentration (µg/g dry mass)
Dissolved concentration (µg/L)
0
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
25
30
B)
Tissue concentration (µg/g dry mass)
Dissolved concentration (µg/L)
0
5
10
15
20
25
0
0.5
1
1.5
2
2.5
3
3.5
4

Figure 3.3.

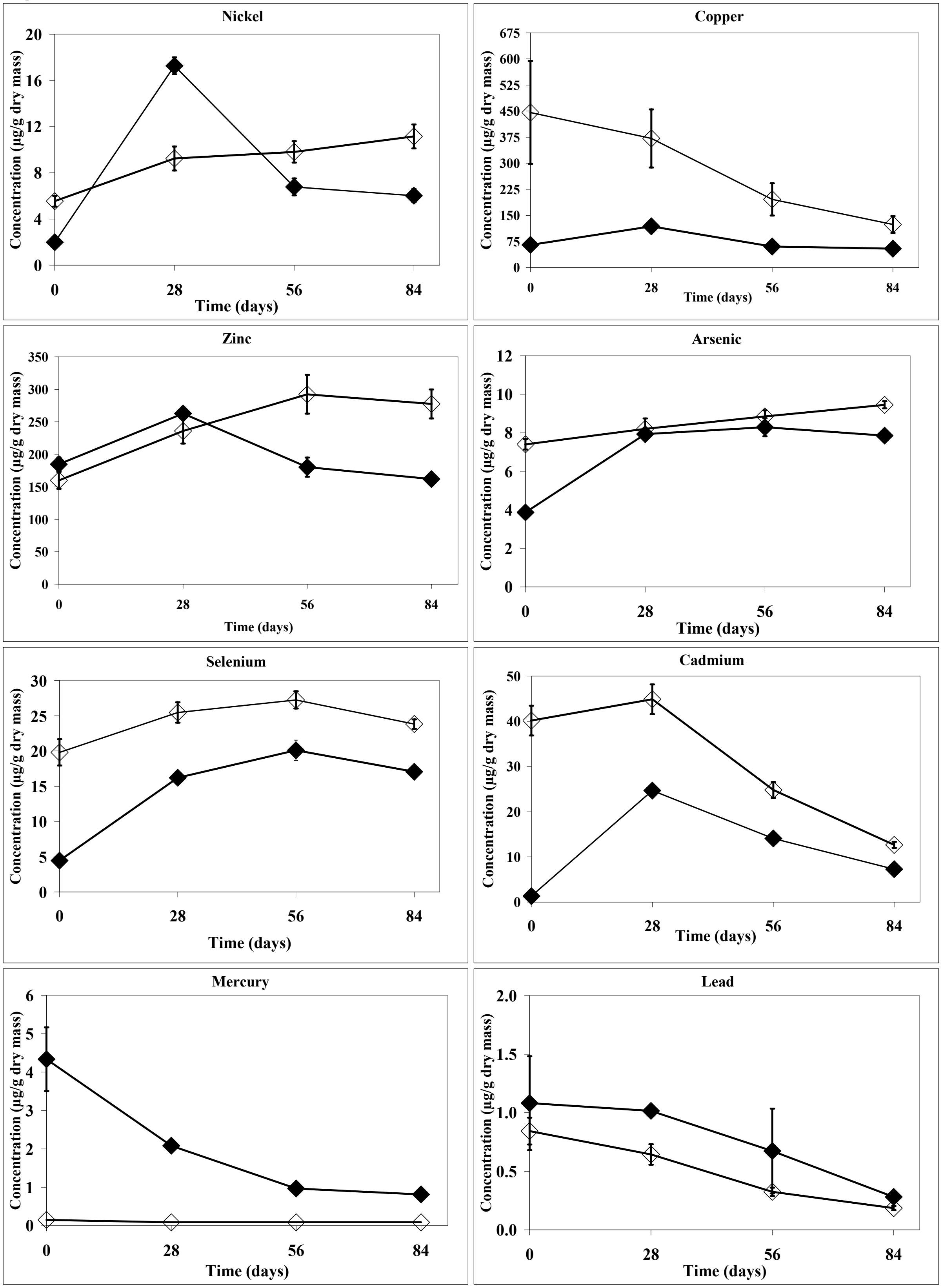

Nickel
Concentration (μg/g dry mass)
Time (days)
Copper
Zinc
Arsenic
Selenium
Cadmium
Mercury
Lead

Figure 3.4

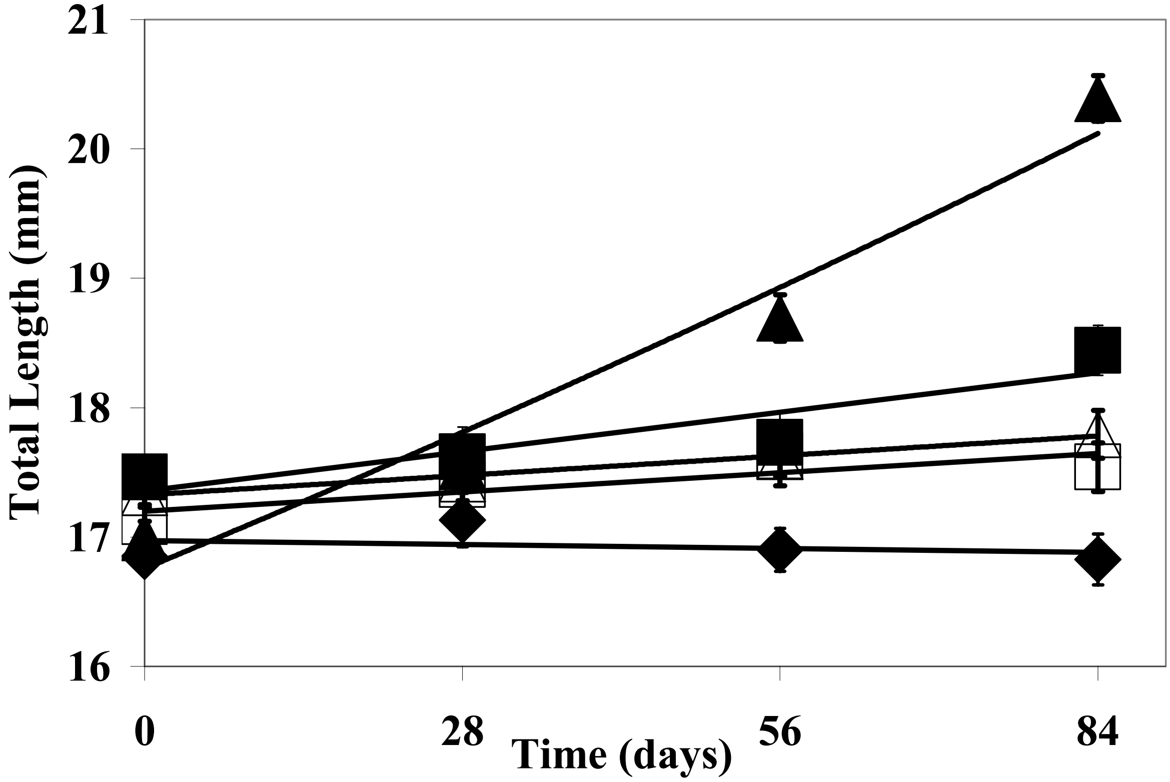


Figure 3.5

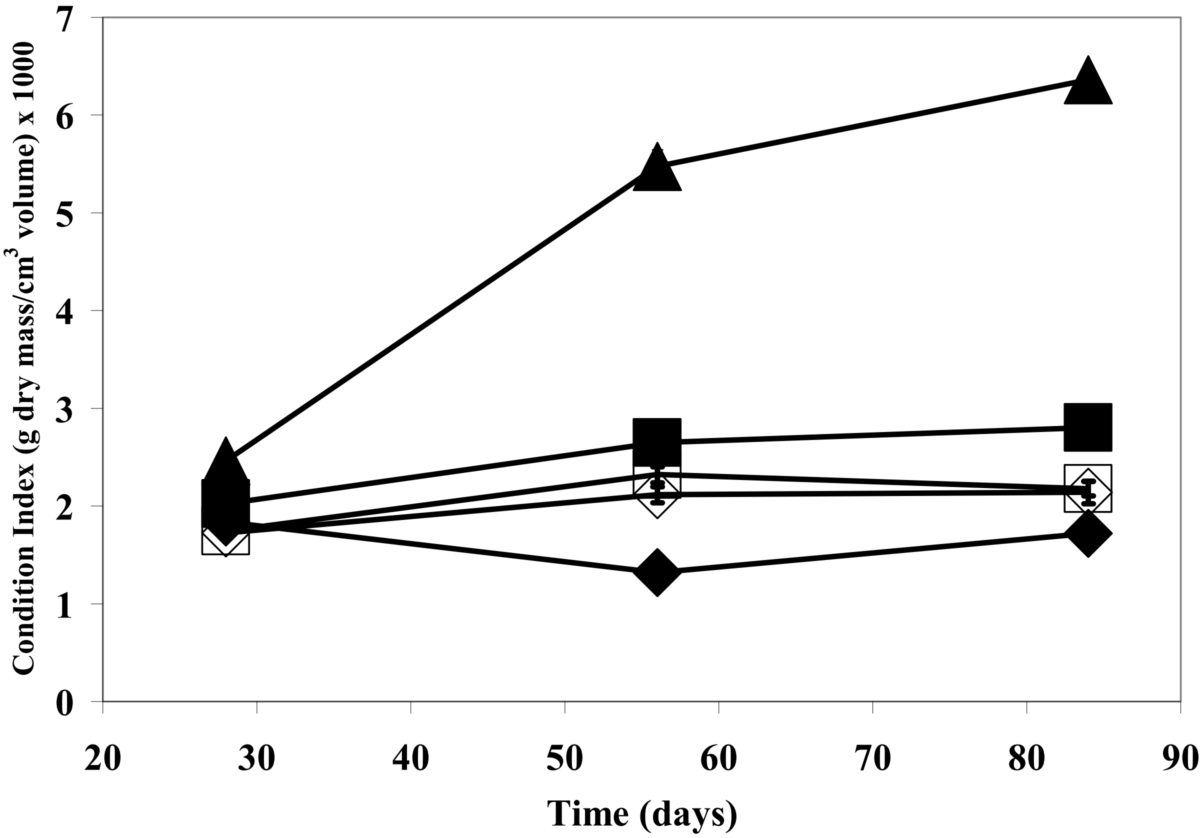

7
6
5
4
3
2
1
0
Condition Index (g dry mass/cm3 volume) x 1000
20
30
40
50
60
70
80
90
Time (days)

Figure 3.6

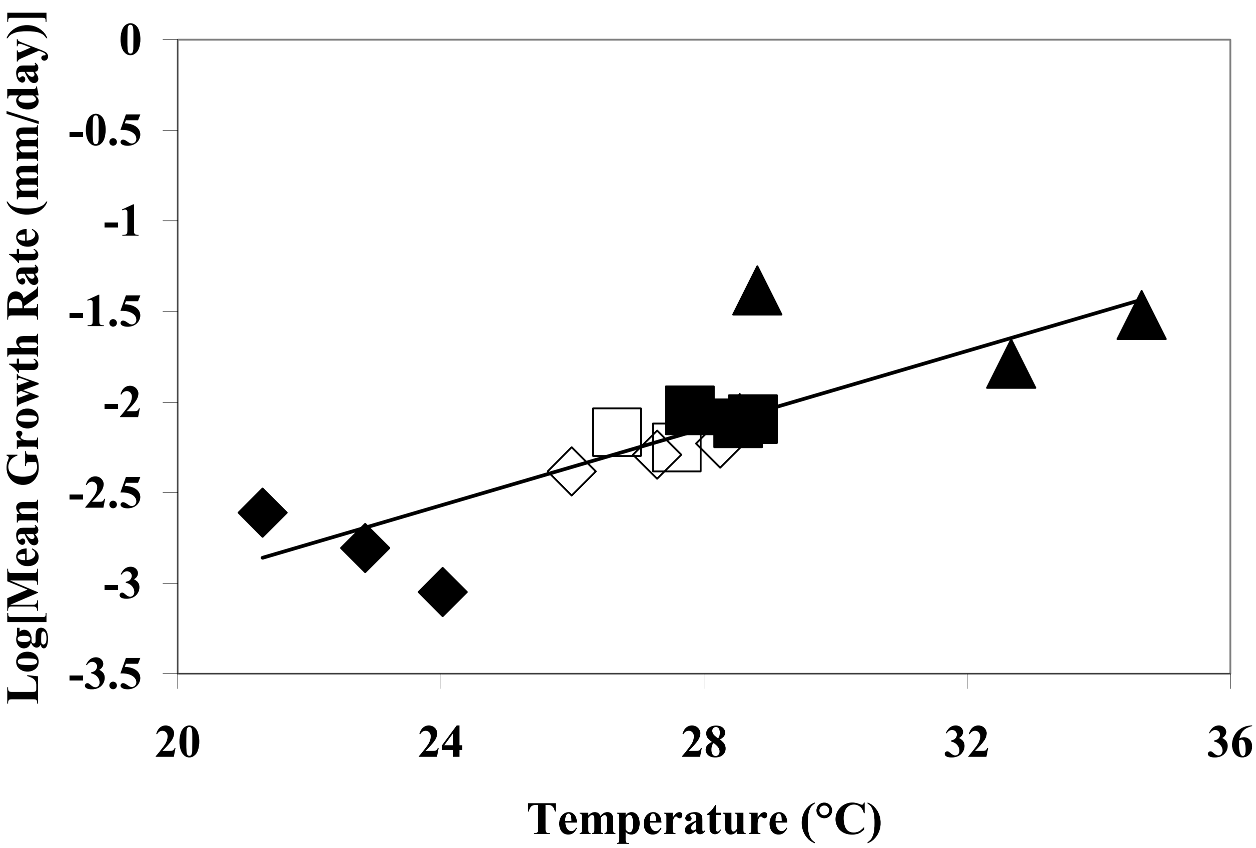

Log[Mean Growth Rate (mm/day)]
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
20
24
28
32
36
Temperature (°C)

Figure 3.7

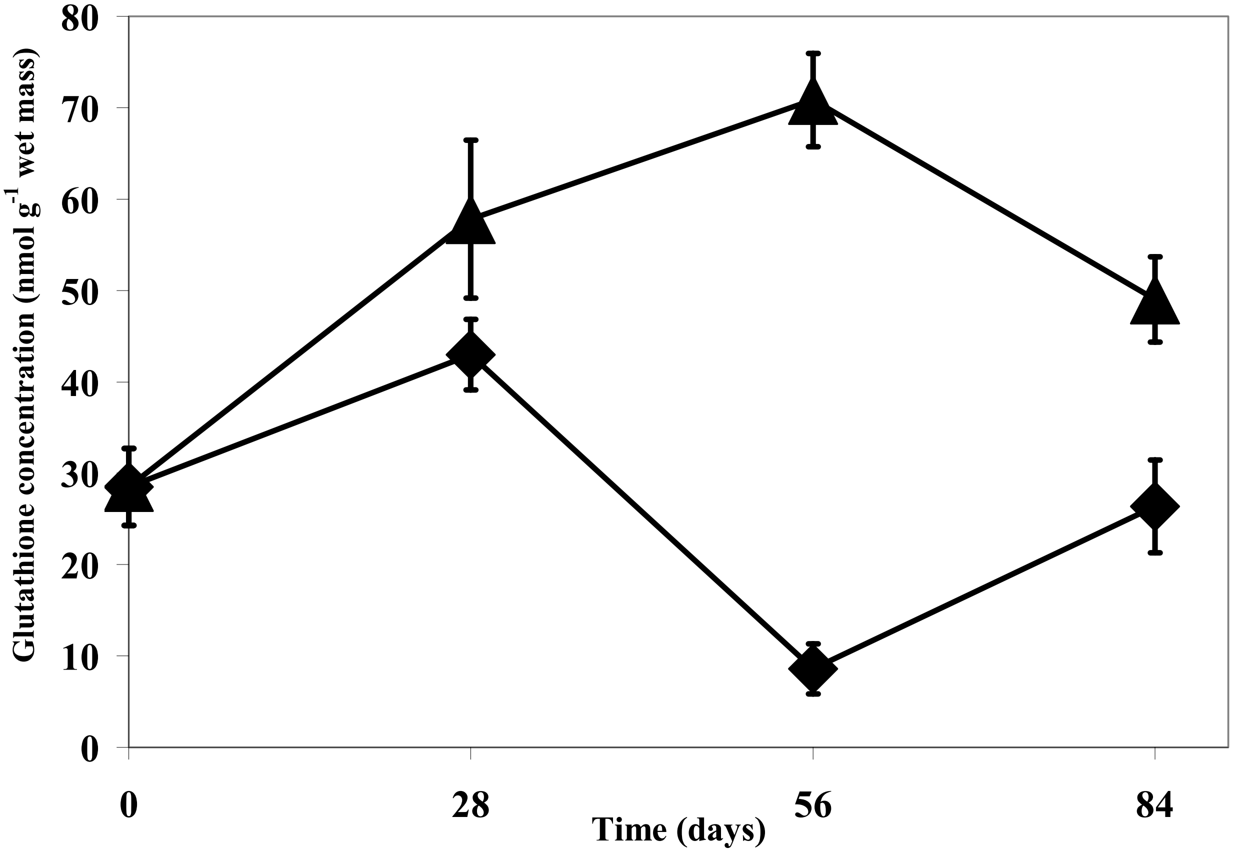
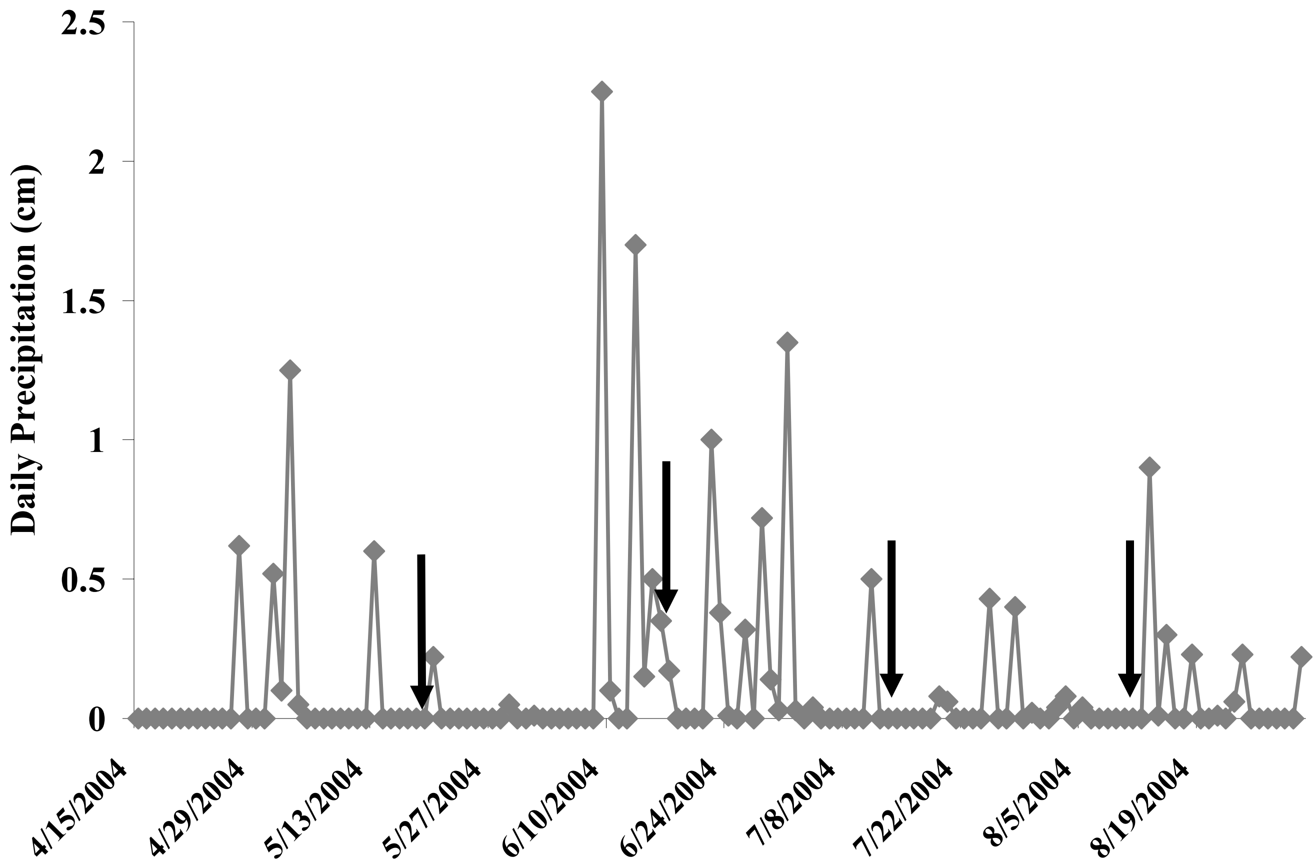

80
70
60
50
40
30
20
10
0
Glutathione concentration (nmol g-1 wet mass)
0
28
56
84
Time (days)

Figure 3.8


2.5
2
1.5
1
0.5
0
Daily Precipitation (cm)
4/15/2004
4/29/2004
5/13/2004
5/27/2004
6/10/2004
6/24/2004
7/8/2004
7/22/2004
8/5/2004
8/19/2004

CHAPTER 4

IS *CORBICULA FLUMINEA* A RELIABLE INDICATOR OF METAL CONTAMINATION IN LOTIC FOOD WEBS?[1]

---

[1]Peltier, Gretchen Loeffler, Judy L. Meyer, William A. Hopkins. To be submitted to *Freshwater Biology*.

Summary

1. Elevated concentrations of selenium, cadmium, and arsenic ("trace elements") in tissues of *Corbicula fluminea* have been observed downstream of discharges from ash-settling basins associated with coal-fired power plants. The objective of this research was to determine if trace element concentrations in *Corbicula* tissue are indicative of metal contamination throughout the aquatic food web. We determined trace element concentrations in tissues from basal food resources, and representative components of the food web (crayfish, clams, macroinvertebrates, and fishes) collected from two streams on the Savannah River Site (one contaminated by discharge from an ash-settling basin and one reference).
2. Selenium concentrations in all invertebrates and fishes from the contaminated stream were significantly higher than those from the reference. For example, concentrations in *Anguilla rostrata* from the contaminated and reference streams were 20.00 ± 1.76 and 2.37 ± 0.22 µg/g dry mass (DM), respectively.
3. Arsenic concentrations were also significantly higher in the contaminated stream. In both streams, concentration in *Gomphus sp.* (25.93 ± 8.51 and 1.55 ± 0.21 µg/g DM in contaminated and reference, respectively) was higher than all other invertebrates and fishes.
4. Cadmium concentrations in all invertebrates and fishes were significantly higher in the contaminated stream. Concentrations in clams were significantly higher than all other food web components in both streams and differed by an order of magnitude between the contaminated stream (26.07 ± 2.54 µg/g DM) and reference (2.36 ± 0.36 µg/g DM).

5. Arsenic, selenium, and cadmium concentrations were highly correlated ($r > 0.50$) in clams, invertebrates, and fishes.
6. Elevated trace element concentrations in *Corbicula* tissue are a reliable indicator of elevated concentrations food web contamination in impacted streams.
7. The food webs (basal resources to generalist fishes) at both sites showed evidence of biomagnification of selenium because concentrations increased with trophic position: selenium concentration and $\delta^{15}N$ were positively correlated ($r^2 = 0.44$, $p<0.0001$ at the contaminated site and $r^2 = 0.21$, $p=0.0008$ at the reference site). Mercury also was biomagnified in the food web at the reference site: concentrations were positively correlated with $\delta^{15}N$ ($r^2=0.33$, $p<0.001$). No evidence of biomagnification was observed at the contaminated site, where mercury concentrations were uniformly low.

Introduction

Increased urbanization is associated with chemical, hydrological, and physical changes in rivers and streams. The term, urban stream syndrome, describes the patterns of chemical, physical, and biological degradation associated with increased urbanization in a catchment (Meyer et al. 2005). One component of the urban stream syndrome is elevated concentrations of metals (e.g. zinc, copper, nickel, cadmium) and metalloids (e.g. arsenic and selenium) (Paul and Meyer 2001), which we collectively term trace elements. These come from various point and non-point sources including coal-fired power plant discharges, urban runoff, and industrial activities (Paul and Meyer 2001, Chapter 2). Typically these inputs occur in combination (e.g., coal-fired power plants often discharge into urban rivers that also receive discharges from wastewater treatment plants), and trace element accumulation in lotic food webs downstream of these multiple sources is not adequately understood (Rowe et al. 2002).

Corbicula fluminea has been widely used as an indicator of contaminants in the USGS National Water Quality Assessment (NAWQA) program and other studies (Graney et al. 1983, Hull et al. 2004, Hull et al. 2006), and concentrations of trace elements in water have been correlated with concentrations in *Corbicula sp.* tissue (Graney et al. 1983). However, to our knowledge, this is the first study to determine if *Corbicula sp.* is an indicator of contamination throughout the food web. *Corbicula* are efficient at removing particles from the water column and are capable of filtering large volumes of water and impacting water clarity and nutrient concentrations (McMahon 1991, Vaughn and Hakenkamp 2001). Clams also have high assimilation efficiencies for some trace elements like zinc, cadmium and selenium (Reinfelder et al. 1997) which are often associated with particles. Because *Corbicula* is particularly efficient at particle removal and trace element assimilation, one cannot be certain that elevated trace elements in *Corbicula* tissues are indicative of elevated concentrations in other lotic organisms. Although dissolved concentrations of trace elements are often used to infer tissue concentrations, the trophic transfer of contaminants through diet has been shown to significantly contribute to trace element accumulation in aquatic organisms (Luoma et al. 1992, Mason et al. 2000, Luoma and Rainbow 2005). Therefore the ability to predict food web contamination using a widespread indicator organism like *Corbicula* would be a valuable tool for both scientists and managers.

Contaminant concentrations can vary with trophic position; the most familiar example is biomagnification of mercury in aquatic food webs. Stable isotopes can be used to determine trophic structure in food webs (Vander Zanden and Rasmussen 1999, Vander Zanden et al. 1999), which can be combined with contaminant analyses to determine if specific species or dietary links are vulnerable to contamination. Stable nitrogen isotope ratios are enriched at each trophic level by ~3.4‰ and can be used to determine trophic position of a species in the food web (Minagawa and Wada 1984, Cabana and Rasmussen 1994). Isotopes can also be used to trace pathways of organic matter from different sources. If carbon sources have distinct isotopic signatures, $\delta^{13}C$ values can be used to differentiate among food resources because little fractionation of carbon occurs between trophic levels (Deniro and Epstein 1978). Integration of stable isotope and contaminant analyses has been useful in demonstrating biomagnification of trace elements such as selenium (Barwick and Maher 2003, Stewart et al. 2004). In San Francisco Bay, two distinct food webs (clam-based vs. crustacean-based) were identified using carbon isotopes. Both showed evidence of selenium biomagnification using nitrogen isotopes to identify trophic levels, but the clam-based food web accumulated higher concentrations of selenium at each trophic level than the crustacean-based food web (Stewart et al. 2004).

The first objective of this study is to determine whether trace element concentrations in *Corbicula fluminea* tissue are indicative of trace element contamination throughout the stream food web. We test the hypothesis that trace elements associated with discharges from coal-fired power plants are not only accumulating in *Corbicula fluminea* but also are accumulating in representative components of the food web. Trace element concentrations in *Corbicula fluminea* were elevated downstream of coal-fired power plant discharges in the Chattahoochee River and on the Savannah River Site (Chapters 2 and 3). We predict that concentrations of trace elements also will be significantly higher in basal resources (algal and detrital) and representative components of the lotic food web (filtering, omnivorous, and predatory invertebrates and generalist insectivore fishes) at Beaver Dam Creek, a stream receiving effluent from a coal-fired power plant at the Savannah River Site compared to Meyers Branch, a reference stream. Under ideal conditions, one would compare concentrations in predatory, benthic, and water column fishes; however, we were limited by the fish assemblages present in both streams and hence can only compare generalist invertivores. We anticipate that mercury concentrations will be an exception to this general pattern. *Corbicula fluminea* in Beaver Dam Creek had significantly lower mercury concentrations than clams from Meyers Branch because of mercury's antagonistic relationship with selenium (Chapter 3), and we expect to observe the same relationship in other food web components in these streams.

The second objective of this study is to examine patterns of correlation among trace elements. Concentrations of nickel, copper, zinc, arsenic, selenium, and cadmium were positively correlated in clams whereas mercury was negatively correlated with selenium and arsenic (Chapter 3); we predict concentrations of these elements will also be correlated in invertebrates and fishes from the two streams. Thirdly, we determine if bioaccumulation and/or biomagnification of trace elements occurs in either stream using stable isotopes to establish trophic position.

Methods

Site Description

We sampled representative components of the aquatic food web over a two-week period in July 2005 on the U.S. Department of Energy's Savannah River Site (SRS) near Aiken, SC. The reference stream, Meyers Branch, is a historically unimpacted blackwater stream on the SRS set-aside for ecological research; it drains approximately 5,085 ha (McArthur and Tuckfield 2000). The contaminated stream, Beaver Dam Creek, is located near a coal-fired power plant on the SRS that has been in operation since 1952. Fly ash and bottom ash from the plant travel through two settling basins, which empty into a 2 ha lentic habitat that is the headwaters of Beaver Dam Creek. Combined water residence time in the series of basins is approximately 2 months (Sandhu et al. 1993). Sampling in Beaver Dam Creek was approximately 30 m downstream of the outlet from the lentic habitat.

Ambient water quality

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 hour at 500°F) 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© turbidmeter (HACH company, Loveland, CO). Collection and analysis of samples for trace elements is described below.

Food resources

Four food resources representing detrital and algal basal resources were sampled at each site: benthic organic matter, conditioned leaves, seston, and biofilm. We collected benthic organic matter (BOM) by elutriating the top 10 cm of sediment, pouring the liquid through a 20 µm plastic mesh, and retaining particles in a whirlpak bag. Conditioned leaves (referred to as coarse particulate organic matter (CPOM) were also collected. To collect seston, a plankton tow net (20µm) was anchored to the stream bottom upstream of the study site and left in place for approximately 20 minutes. Collected particles were rinsed into a whirlpak bag for storage. Biofilm was scraped from the surface of wooden dowels that were anchored at the two study sites one month prior to sampling. If filamentous algae were present, they were sampled separately. Three samples of each food resource were collected from each study site. All samples were frozen (-70°C), freeze-dried, and homogenized prior to trace element and stable isotope analyses.

Invertebrates and clams

Using D-nets, kick seines, and picking leaf packs and woody debris, we sampled the stream bottom and banks at Meyers Branch and Beaver Dam Creek. All invertebrates were placed in aerated site water for 24 hours to clear their guts and then frozen at -70°C. In the laboratory, clams were identified to species, macroinvertebrates to genus, and crayfish to family. Whole organisms were rinsed in deionized-distilled water, weighed (wet mass), frozen, freeze-dried, and homogenized prior to trace element and stable isotope analysis. Asiatic clams (*Corbicula fluminea*) were measured (shell length, width, and depth); tissues removed from shells and treated in the same manner as other invertebrates. We sorted all samples and identified taxa for which we had adequate amounts of tissue for analyses. We compared trace element concentrations in representative filter-feeders (*Corbicula fluminea*), predators (*Gomphus sp.*), and omnivores (Cambaridae) between the two study sites (Table 4.1). Caddisfly genera differed in the two streams: *Macronema sp.*, a filterer, occurred in the contaminated stream, and *Hydatophylax sp.*, a shredder, occurred in the reference stream. Trace element concentrations in their tissues were also determined. For *Corbicula fluminea*, *Hydatophylax sp.*, *Gomphus sp.*, and Cambaridae, we conducted trace element and stable isotope analysis on five individuals from each site. *Macronema sp.* from Beaver Dam Creek were too small to analyze individually so we pooled them into five size classes.

Fishes

Fishes were sampled using a backpack shocker, placed on ice immediately in the field, and frozen until identification and dissection. After thawing, guts were removed and remaining whole bodies were frozen, freeze-dried, and homogenized prior to trace element and stable isotope analysis. Samples from Beaver Dam Creek included only four species of fish: eastern mosquitofish (*Gambusia holbrooki*), American eel (*Anguilla rostrata*), spotted sunfish (*Lepomis punctatus*), and redbreast sunfish (*Lepomis auritus*). Diversity of fishes at Meyers Branch was much higher (14 species) and included American eel (*Anguilla rostrata*), redbreast sunfish (*Lepomis auritus*), creek chub (*Semotilus atromaculatus*), yellow fin shiner (*Notropis lutipinnis*). We focused on feeding guild comparisons between the two sites rather than species comparisons because the two streams only had adequate numbers of one fish species (American eel) in common (Table 4.1). Five similarly-sized individual fish were analyzed for each feeding guild comparison (generalist, generalist insectivore with small mouth, generalist insectivore with large mouth) from each study site. Similar sizes were used to minimize the effect of size and age on trace element comparisons. We also analyzed the two redbreast sunfish collected from Meyers Branch for comparison with redbreast collected from Beaver Dam Creek even though replication was not adequate for statistical analysis.

Trace element sampling and analysis

Water samples were filtered in the field using GHP Acrodisc GF 25 mm syringe filters with GF/0.45 µm GHP membranes (Pall Life Sciences, East Hills, NY, USA) for dissolved trace element concentrations. Filtered samples were acidified with trace-metal grade nitric acid prior to freezing. Field blanks using deionized-distilled water were treated in the same manner as filtered water samples.

Approximately 20-60 mg dry mass (DM) of clams, dragonflies, and caddisflies, and 150-200 mg DM of food resources, crayfish, and fishes were used for digestion. Trace metal grade nitric acid (3 ml and 5 ml) was added to the samples prior to digestion in a microwave (MDS

2000, Matthews, NC, USA). Samples were brought to a final volume of 10 ml or 15 ml with deionized-distilled water. All trace element analyses were performed using an inductively coupled plasma mass spectrometer (ICP-MS) (Perkin Elmer, Norwalk, CT). We measured total concentrations of vanadium (V), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), cadmium (Cd), mercury (Hg), and lead (Pb). Concentrations of V, Mn, Fe, Co, and Sr are not discussed further, but all of the trace element concentrations for tissues and water are reported in Appendix C. Certified reference materials of an appropriate matrix (Riverwater, MESS-2, DORM, and Tort 2; NRC, Ottawa, Canada) and blanks were included in the digestion and analysis procedure. Mean percent recoveries ranged from 88 – 124%.

Stable isotope analysis

We conducted stable isotope analysis (^{15}N and ^{13}C) on all food resources, invertebrates, clams, and fishes. Sub-samples of 10 mg DM (BOM and seston) and 1-2 mg DM (all other samples) were analyzed in a Thermo Finnigan DeltaPlus mass-spectrophotometer (Thermo Finnigan) at the Institute of Ecology, University of Georgia. The standard reference material for carbon was Pee Dee belemnite and atmospheric N_2 for nitrogen.

Statistical analyses

We tested the normality of trace element concentrations at each site with the Shapiro-Wilk's test and transformed them using $\log_{10}(x+1)$. A t-test was used to determine if trace element concentrations were significantly different in *Corbicula* from the two study streams. Within a study site, one-way ANOVAs were used to compare trace element concentrations of food web components followed by Tukey-Kramer post hoc tests. We used three two-way ANOVAs to compare trace element concentrations among the food webs components from the two streams. The first two-way ANOVA (site*basal resource) compared food resources within and between streams (4 groups; each group has N=3). The second two-way ANOVA (site*invertebrate) compared all invertebrates within and between streams (3 groups; each group has N=5). The third two-way ANOVA (site*fishes) compared feeding guilds of fishes within and between streams (3 groups; each group has N=5). If there were significant interaction effects for the two-way ANOVA comparisons, we conducted a series of t-tests to compare component concentrations (e.g. seston and seston) between the two streams.

Pearson's correlation coefficient (*r*) was used to explore relationships among trace elements in tissues from the two study streams. Simple linear regression was used to relate trace element concentrations and trophic level ($\delta^{15}N$ values) in the entire food web from each stream. Significant positive relationships between trace element concentration and $\delta^{15}N$ values provide evidence for biomagnification. JMP 5.0.1 or SAS 9.1 (SAS Institute, Cary, NC, USA) were used for statistical analyses.

Results

Ambient water quality

The two streams displayed distinct differences in specific conductance, temperature, and DOC concentration (Table 4.2). Specific conductance and temperature were higher at Beaver Dam Creek, whereas DOC concentration was highest at Meyers Branch (Table 4.2). The streams did not differ in dissolved oxygen or pH. Alkalinity and hardness were both 27 mg $CaCO_3$/L at Meyers Branch, but at Beaver Dam Creek, hardness was slightly higher (31 mg $CaCO_3$/L) and alkalinity slightly lower (22 mg $CaCO_3$/L) (Table 4.2). Dissolved concentrations of Ni, Cu, Zn, As, Se, and Cd were also higher at Beaver Dam Creek (Table 4.2). These differences in water quality between the two study sites were consistent with observations during the previous summer (Chapter 3).

Trace element concentrations in clams

Significant differences in tissue concentrations of *Corbicula fluminea* from the two study sites were evident and consistent with results from an earlier study (Chapter 3). Clams from Beaver Dam Creek had significantly higher concentrations of Ni (t-test, $p < 0.0001$), Cu ($p = 0.0012$), Zn ($p = 0.04$), As ($p < 0.0001$), Se ($p < 0.0001$), and Cd ($p < 0.0001$); and clams from Meyers Branch had significantly higher concentrations of Hg ($p < 0.0001$) (Table 4.3). Concentrations of Pb in clam tissue were not significantly different between the two streams (Table 4.3).

Food web structure

The food web in the two streams had three distinct trophic levels (basal resources, primary consumers, and secondary consumers) with an increase in $\delta^{15}N$ of 2-4‰ between trophic levels (Figures 4.1 and 4.2). Three of the *Gomphus sp.* individuals from Meyers Branch were significant outliers with inexplicably high negative $\delta^{13}C$ signatures (-38 to -41‰), and we have omitted them from further analyses. Using carbon signatures ($\delta^{13}C$), we found no evidence of separate food webs supported by different basal resources (e.g., algal vs. detrital, Figures 4.1 and 4.2). Nitrogen isotopes at each trophic level were enriched ~2-3‰ at Beaver Dam Creek compared to Meyers Branch (Figure 4.1 vs. Figure 4.2), which may reflect wastewater treatment plant discharges (Heikoop et al. 2000, Costanzo et al. 2001) upstream of the coal-fired power plant's intake from the Savannah River.

Differences between streams in trace element concentrations in food webs

Trace element concentrations in all components of the food web differed significantly between streams. Trace element concentrations of Ni, Cu, Zn, As, Se, and Cd were significantly higher (two-way ANOVA and t-test, $p < 0.05$) in benthic organic matter, biofilm, and seston from Beaver Dam Creek than Meyers Branch (Table 4.3). Cu, As, and Cd concentrations at Beaver Dam Creek were over an order of magnitude higher than at Meyers Branch; similarly, concentrations of Ni, Zn, and Se at Beaver Dam Creek were 5-10 times higher (Table 4.3).

Trace element concentrations in all invertebrates were significantly higher (two-way ANOVA and t-test, $p < 0.05$) at Beaver Dam Creek than Meyers Branch for all elements except Zn, Hg, and Pb (Table 4.3). Zn concentrations in *Hydatophylax sp*. were significantly higher at Meyers Branch (162.9 ± 16.36 µg/g DM) than *Macronema sp.* at Beaver Dam Creek (109.54 ± 9.39 µg/g DM) (Table 4.3). Hg concentrations in *Gomphus* and crayfish at Meyers Branch were ten times higher than *Gomphus* and crayfish from Beaver Dam Creek (Table 4.3). Pb concentrations in invertebrates did not significantly differ between the two streams.

Concentrations of As, Se, and Cd in fishes were significantly higher (two-way ANOVA and t-test, $p < 0.05$) in Beaver Dam Creek, whereas Hg concentrations ($p<0.05$) were significantly higher in fishes from Meyers Branch (Table 4.3). As, Se, and Cd concentrations in fishes at Beaver Dam Creek were an order of magnitude higher than at Meyers Branch. Zn concentrations in fishes were not significantly different between the streams (Table 4.3). Concentrations of Ni, As, Se, and Cd in *Anguilla rostrata* and *Lepomis auritus* were 3-10 times higher at Beaver Dam Creek than Meyers Branch (Table 4.3).

Within-stream differences among food web components

Trace element concentrations within the three groups measured (basal resource, invertebrate, or fish) differed. In both streams, Ni and Hg concentrations in all four basal resources were not significantly different (ANOVA, $p > 0.05$); however for all other trace elements there was a lack of consistent response among resources (Table 4.3). Concentrations of Cu, Zn, As, Se, and Cd in biofilm, benthic organic matter, and seston were statistically similar ($p > 0.05$) at Beaver Dam Creek (Table 4.3). Concentrations of Ni, Cu, Se, and Hg were similar in all basal resources at Meyers Branch (Table 4.3).

Body burdens of trace elements differed among invertebrate consumers. The invertebrate predator, *Gomphus sp.,* had higher body burdens of As and Hg than crayfish and caddisflies (Table 4.3). Concentrations of Ni, Zn, Se, and Hg did not significantly differ ($p > 0.05$) in crayfish, caddisflies, or dragonflies at Beaver Dam Creek (Table 4.3). Similarily, Ni, Cu, Cd, and Hg concentrations did not differ among invertebrates at Meyers Branch (Table 4.3).

Within both streams, concentrations of Cu, As, Se, Cd, Hg, and Pb did not significantly differ ($p > 0.05$) among fish feeding guilds (Table 4.3), but differences existed for the other two trace elements. Generalists with smaller mouths (*Notropis lutipinnis* and *Gambusia holbrooki*) had significantly higher body burdens ($p < 0.05$) of Ni and Zn than the other two feeding guilds (Table 4.3).

Comparing trace element concentrations in clams to other food web components

Trace element concentrations of Ni, Cu, As, Se, and Cd in *Corbicula* were significantly higher at Beaver Dam Creek than Meyers Branch and these differences were seen in other components of the food web (Table 4.3). For each trace element analyzed, clams from Beaver Dam Creek had statistically similar concentrations to several invertebrates and fishes, but these relationships differed for each element. Clam and generalist-smallmouth fishes did not significantly differ ($p > 0.05$) in body burdens of Ni, Zn, and Se (Table 4.3). *Corbicula, Gomphus sp*., and *Macronema sp.* had similar ($p > 0.05$) concentrations of Ni, As, Se, and Pb

(Table 3). For two trace elements, concentrations in clams were significantly higher than in any of the food web components measured. Cd concentrations in clams (26 µg/g DM) were significantly higher ($p < 0.05$) than all the other components which ranged from 0.41-15 µg/g DM (Table 4.3). At Meyers Branch, Hg concentrations in clams (4.89 µg/g DM) were significantly higher than all other food web components, which ranged from 0.02-1.00 µg/g DM (Table 4.3).

Correlations among trace elements

Strong positive correlations ($r \geq 0.70$) among all trace element concentrations except Hg and Pb existed in *Corbicula.* Mercury concentrations in clams were negatively correlated ($r \geq 0.80$) with Ni, As, Se, and Cd. We expected that these same relationships would be observed in invertebrates and fishes. Ni, As, Se, and Cd were positively correlated ($r \geq 0.50$) among invertebrates, consistent with relationships observed in clams; however, strong negative correlations among trace elements did not occur (Table 4.4). Among fishes, As was positively correlated ($r \geq 0.80$) with Se and Cd whereas Hg concentrations were negatively correlated ($r \geq 0.50$) with As, Se, and Cd (Table 4.5). In clam, invertebrate, and fish comparisons, As, Se, and Cd were positively correlated (Tables 4.4 and 4.5). However among the other trace elements, negative correlations observed in clams and fishes were not evident in invertebrates (Tables 4.4 and 4.5).

Evidence for biomagnification

We did not find evidence for distinct food webs that depend on different basal resources ($\delta^{13}C$ values) in either stream, so all components of the food web were included in analyses of biomagnification. Se concentrations in the food web (food resources, invertebrates, and fishes) were significantly positively correlated with ^{15}N values at Beaver Dam Creek (r^2=0.44, p<0.0001), which implies biomagnification of selenium in the stream food web. Biomagnification of selenium was also suggested in the Meyers Branch food web ($r^2 = 0.21$, $p = 0.0008$), but the regression explained a much smaller fraction of the variation in Se concentration, although the range in concentration was much less (Figure 4.3). Biomagnification of mercury was not evident in either stream when all food web components were included in the analysis. However, if clams were removed, mercury concentration in the food web was positively correlated with $\delta^{15}N$ ($r^2 = 0.43$, $p < 0.001$) at Meyers Branch, implying biomagnification. None of the other trace element concentrations were positively correlated with ^{15}N values in either stream. At Beaver Dam Creek, concentrations of all trace elements (except mercury) were negatively correlated with increasing trophic level.

Discussion

Differences between streams

Both trace element concentrations (except Hg) in components of the food web and dissolved concentrations were significantly higher in Beaver Dam Creek than Meyers Branch. Concentrations of As, Se, and Cd were significantly higher in basal resources, invertebrates, and fishes from Beaver Dam Creek (Table 4.3), and these trace elements are abundant in coal combustion waste (Rowe et al. 2002). Concentrations of Se in all three invertebrates (13-18 µg/g DM) from Beaver Dam Creek exceeded the proposed dietary Se threshold of 3 µg/g DM for fish (Lemly 1993a, Hamilton 2002, 2004). Similarly, Se concentrations in fishes from Beaver Dam Creek (20-21 µg/g DM) exceeded the whole-body toxicity threshold of 4 µg/g DM based on field and laboratory studies of other fish species (Hamilton 2002, 2004).

Mercury concentrations in clams and other food web components were significantly higher in Meyers Branch than Beaver Dam Creek. Atmospheric deposition contributes the majority of mercury inputs into aquatic systems (Mason et al. 2000), which should not differ between the two streams. However, Meyers Branch is a southeastern coastal blackwater stream, and its higher humic acid content may lead to methylation of mercury (Ravichandran 2004). DOC concentrations were also higher in Meyers Branch (Table 4.2), and total and methylmercury concentrations increased in lakes with increasing DOC concentrations (Driscoll et al. 1995). Higher DOC concentrations may lead to higher Hg transport and higher concentrations in the water column for aquatic organisms.

Correlations among trace elements

Mercury was highly negatively correlated with several trace elements in fishes (As, Se, Cd), but not in invertebrates. We measured total mercury concentrations in tissues, but in the muscle tissues of freshwater fishes and marine invertebrates >95% of mercury is in the form of methylmercury (MeHg) (Bloom 1992). Total Hg and total Se have been weakly inversely correlated in many aquatic organisms, but much stronger inverse correlations have been shown for MeHg and total Se (Belzile et al. 2006). The differences in mercury correlations in invertebrates and fishes (Tables 4.4 and 4.5) may be a result of differences in the chemical form of mercury in freshwater fishes versus freshwater invertebrates. Differences in mercury correlations may also be related to physiological differences between invertebrates and fishes.

Evidence for biomagnification

Two trace elements (Se and Hg) showed evidence of biomagnification from basal resources to generalist fishes through the food web. Selenium concentrations in clams and all components of the food web were significantly higher at Beaver Dam Creek compared to Beaver

Dam Creek (ANOVA, $p<0.0001$). However, we observed biomagnification of selenium in the food webs of both streams (Figure 4.3). Studies in estuarine systems (Barwick and Maher 2003, Stewart et al. 2004) and lentic environments (Simmons and Wallschlager 2005, Orr et al. 2006) have also observed biomagnification of selenium, but few studies have observed it in lotic environments (Simmons and Wallschlager 2005, Orr et al. 2006). Biomagnification of mercury has been observed in many freshwater and marine environments (e.g., Cabana and Rasmussen 1994, Atwell et al. 1998, Morel et al. 1998, Campbell et al. 2005), but mercury biomagnification was detectable at Meyers Branch only when clams were removed from the analysis. Mercury concentrations in Beaver Dam Creek were uniformly low. None of the other trace elements showed evidence of biomagnification in either stream.

Are clams reliable indicators of trace element contamination in lotic food webs?

Tissue concentrations in clams reflect the differences in trace element concentrations in other components of the stream food webs. In the contaminated stream, only Cd concentrations in clams were significantly higher than other food web components, and clams have been shown to have high assimilation efficiencies for Cd (Reinfelder et al. 1997). For all other trace elements in Beaver Dam Creek, concentrations in clams were statistically similar to some of the basal resources, invertebrates, and fishes. Some invertebrates and fishes from Beaver Dam Creek had statistically similar body burdens of all trace elements (except Cd) compared to clams. However, for each trace element, the type of invertebrate or fish that was similar to *Corbicula* (e.g., predator or generalist with small mouth) differed. In Meyers Branch, the reference stream, concentrations of fewer trace elements in clams were similar to concentrations in other food web components. Se, Cd, and Hg concentrations in clams were significantly higher than all other food web components. Concentrations of Ni and Zn in clams were statistically similar to invertebrates and fishes in Meyers Branch, whereas Cu and As were similar to invertebrates and basal resources, respectively. *Corbicula fluminea* may be a useful tool for predicting food web contamination in highly contaminated streams, but its utility appears to be limited in unimpacted, reference streams.

To further pursue the idea of using clams as indicators, we asked whether food web contamination is likely in an urban river. We compared concentrations in *Corbicula fluminea* from several sites in the Chattahoochee River basin to clams in this study to determine if the potential for food web contamination exists, particularly at sites near coal-fired power plant (CFPP) discharges (Table 4.6). Based on the concentrations of As and Se in clams, one would expect elevated concentrations in invertebrates and fishes from the three CFPP sites in the Chattahoochee River (Table 4.6). Cu and Zn concentrations may be elevated in invertebrates and fishes from both urban and CFPP sites in the river (Table 4.6).

Conclusion

Corbicula fluminea are often used as indicators of contamination in rivers and lakes because they are filter-feeders, able to tolerate changes in environmental conditions, and found throughout urban and rural catchments. We sought to determine whether *Corbicula fluminea* could be used as an indicator of contamination throughout a lotic food web. Clams from a stream contaminated with CFPP discharges had elevated concentrations of trace elements compared to clams from a reference stream. The basal resources, invertebrates, and fishes collected from the contaminated stream also had elevated concentrations of trace elements. In the contaminated stream, trace element concentrations in *Corbicula fluminea* were similar to concentrations in invertebrates and fishes for all elements except Cd. In the reference stream, Ni, Cu, Zn, and As concentrations in clams were similar to concentrations in other food web components. In both streams, Se biomagnified from basal resources to generalist fishes in the food web. Clams are reliable indicators of food web contamination in impacted streams and provide a simple tool for monitoring and assessment of impacted streams.

Acknowledgements

We would like to thank Jason Unrine and the Advanced Analytical Center for Environmental Sciences at the Savannah River Ecology Laboratory for assistance in ICP-MS analysis. Craig Baker, Rebecca Coleman, Will Duncan, Gabrielle Graeter, Ashley Helton, Cindy Tant, and Meredith Wright assisted with field sampling. Stable isotopes were analyzed by Tom Maddox at the Institute of Ecology, University of Georgia. Special thanks to David Walters, Robin Stewart, and Jason Unrine for guidance in the design of the food web study.

Literature Cited

Atwell, L., K. A. Hobson, and H. E. Welch. 1998. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. Canadian Journal of Fisheries and Aquatic Sciences **55**:1114-1121.

Barwick, M., and W. Maher. 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**:471-502.

Belzile, N., Y. W. Chen, J. M. Gunn, J. Tong, Y. Alarie, T. Delonchamp, and C. Y. Lang. 2006. The effect of selenium on mercury assimilation by freshwater organisms. Canadian Journal of Fisheries and Aquatic Sciences **63**:1-10.

Bloom, N. S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Canadian Journal of Fisheries and Aquatic Sciences **49**:1010-1017.

Cabana, G., and J. B. Rasmussen. 1994. Modeling food-chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature **372**:255-257.

Campbell, L. M., R. J. Norstrom, K. A. Hobson, D. C. G. Muir, S. Backus, and A. T. Fisk. 2005. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). Science of the Total Environment **351**:247-263.

Costanzo, S. D., M. J. O'Donohue, W. C. Dennison, N. R. Loneragan, and M. Thomas. 2001. A new approach for detecting and mapping sewage impacts. Marine Pollution Bulletin **42**:149-156.

Deniro, M. J., and S. Epstein. 1978. Influence of diet on distribution of carbon isotopes in animals. Geochimica Et Cosmochimica Acta **42**:495-506.

Driscoll, C. T., V. Blette, C. Yan, C. L. Schofield, R. Munson, and J. Holsapple. 1995. The role of dissolved organic-carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes. Water Air and Soil Pollution **80**:499-508.

Graney, R. L., D. S. Cherry, and J. Cairns. 1983. Heavy-metal indicator potential of the asiatic clam (Corbicula-fluminea) in artificial stream systems. Hydrobiologia **102**:81-88.

Hamilton, S. J. 2002. Rationale for a tissue-based selenium criterion for aquatic life. Aquatic Toxicology **57**:85-100.

Hamilton, S. J. 2004. Review of selenium toxicity in the aquatic food chain. Science of the Total Environment **326**:1-31.

Heikoop, J. M., M. J. Risk, A. V. Lazier, E. N. Edinger, J. Jompa, G. V. Limmon, J. J. Dunn, D. R. Browne, and H. P. Schwarcz. 2000. Nitrogen-15 signals of anthropogenic nutrient loading in reef corals. Marine Pollution Bulletin **40**:628-636.

Hull, M. S., D. S. Cherry, and T. C. Merricks. 2004. Effect of cage design on growth of transplanted Asian clams: Implications for assessing bivalve responses in streams. Environmental Monitoring and Assessment **96**:1-14.

Hull, M. S., D. S. Cherry, and R. J. Neves. 2006. Use of bivalve metrics to quantify influences of coal-related activities in the Clinch River watershed, virginia. Hydrobiologia **556**:341-355.

Lemly, A. D. 1993. Guidelines for evaluating selenium data from aquatic monitoring and assessment studies. Environmental Monitoring and Assessment **28**:83-100.

Luoma, S. N., C. Johns, N. S. Fisher, N. A. Steinberg, R. S. Oremland, and J. R. Reinfelder. 1992. Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. Environmental Science & Technology **26**:485-491.

Luoma, S. N., and P. S. Rainbow. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environmental Science & Technology **39**:1921-1931.

Mason, R. P., J. M. Laporte, and S. Andres. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. Archives of Environmental Contamination and Toxicology **38**:283-297.

McArthur, J. V., and R. C. Tuckfield. 2000. Spatial patterns in antibiotic resistance among stream bacteria: Effects of industrial pollution. Applied and Environmental Microbiology **66**:3722-3726.

McMahon, R. F. 1991. Mollusca: Bivalvia. *in* J. H. Thorp and A. P. Covich, editors. Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego.

Meyer, J. L., M. J. Paul, and W. K. Taulbee. 2005. Stream ecosystem function in urbanizing landscapes. Journal of the North American Benthological Society **24**:602-612.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of N-15 along food-chains - Further evidence and the relation between delta-N-15 and animal age. Geochimica Et Cosmochimica Acta **48**:1135-1140.

Morel, F. M. M., A. M. L. Kraepiel, and M. Amyot. 1998. The chemical cycle and bioaccumulation of mercury. Annual Review of Ecology and Systematics **29**:543-566.

Orr, P. L., K. R. Guiguer, and C. K. Russel. 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. Ecotoxicology and Environmental Safety **63**:175-188.

Paul, M. J., and J. L. Meyer. 2001. Streams in the urban landscape. Annual Review of Ecology and Systematics **32**:333-365.

Ravichandran, M. 2004. Interactions between mercury and dissolved organic matter - a review. Chemosphere **55**:319-331.

Reinfelder, J. R., W. X. Wang, S. N. Luoma, and N. S. Fisher. 1997. Assimilation efficiencies and turnover rates of trace elements in marine bivalves: a comparison of oysters, clams and mussels. Marine Biology **129**:443-452.

Rowe, C. L., W. A. Hopkins, and J. D. Congdon. 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: A review. Environmental Monitoring and Assessment **80**:207-276.

Sandhu, S. S., G. L. Mills, and K. S. Sajwan. 1993. Leachability of Ni, Cd, Cr, and As from coal ash impoundments of different ages on the Savannah River Site. *in* R. K. Keefer and K. S. Sajwan, editors. Trace elements in coal and coal combustion residues. CRC Press, Boca Raton, FL.

Simmons, D. B. D., and D. Wallschlager. 2005. A critical review of the biogeochemistry and ecotoxicology of selenium in lotic and lentic environments. Environmental Toxicology and Chemistry **24**:1331-1343.

Stewart, A. R., S. N. Luoma, C. E. Schlekat, M. A. Doblin, and K. A. Hieb. 2004. Food web pathway determines how selenium affects aquatic ecosystems: A San Francisco Bay case study. Environmental Science & Technology **38**:4519-4526.

Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumer delta C-13 and delta N-15 and the trophic position of aquatic consumers. Ecology **80**:1395-1404.

Vander Zanden, M. J., B. J. Shuter, N. Lester, and J. B. Rasmussen. 1999. Patterns of food chain length in lakes: A stable isotope study. American Naturalist **154**:406-416.

Vaughn, C. C., and C. C. Hakenkamp. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology **46**:1431-1446.

Table 4.1. Food web components collected from the two study sites

Meyers Branch (reference)	**Beaver Dam Creek (coal)**
Food resources	*Food resources*
Conditioned leaves (CPOM)	Conditioned leaves (CPOM)
Benthic organic matter (BOM)	Benthic organic matter (BOM)
Biofilm	Biofilm
Seston	Seston
Invertebrates	*Invertebrates*
Filter-feeder (*Corbicula fluminea*)	Filter-feeder (*Macronema sp., Corbicula fluminea*)
Predator (*Gomphus sp.*)	Predator (*Gomphus sp.*)
Omnivore (Cambaridae)	Omnivore (Cambaridae)
Shredder (*Hydatophylax sp.*)	
Fishes	*Fishes*
Generalist (Anguilla rostrata)	Generalist (*Anguilla rostrata*)
Generalist insectivore with large mouth (*Semotilus atromaculatus* and *Lepomis auritus*)	Generalist insectivore with large mouth (*Lepomis auritus*)
Generalist insectivore with small mouth (*Notropis lutipinnis*)	Generalist insectivore with small mouth (*Gambusia holbrooki*)

Table 4.2. Water quality parameters when organisms were collected. Dissolved organic carbon, alkalinity, hardness, turbidity, and dissolved concentrations of trace elements are mean values ± SE (N=3). BDL = below detection limit.

	Study site	
	Meyers Branch	**Beaver Dam Creek**
Temperature (°C)	22.83	28.91
pH	7.04	7.06
Specific conductance (ms/cm)	0.059	0.195
Dissolved oxygen (mg/L)	7.8	8.46
Dissolved organic carbon (mg/L)	4.65	2.13
Alkalinity (mg $CaCO_3$/L)	27.00 ± 0.00	22 ± 0.00
Hardness (mg $CaCO_3$/L)	27.00 ± 1.67	31 ± 0.67
Turbidity (NTU)	9.37 ± 0.60	2.89 ± 0.12
Ni (μg/L)	0.16 ± 0.01	5.16 ± 0.23
Cu (μg/L)	0.59 ± 0.14	3.30 ± 0.35
Zn (μg/L)	17.15 ± 10.45	23.25 ± 9.59
As (μg/L)	0.44 ± 0.09	11.55 ± 0.46
Se (μg/L)	0.26 ± 0.02	3.31 ± 0.22
Cd (μg/L)	BDL	0.25 ± 0.01

Table 4.3. Mean trace element concentrations (± 1SE) in all food web components from the two study streams expressed in µg/g dry mass. N = number of samples. Significant difference ($p < 0.05$) between streams for each component (e.g. Group B1 from Meyers Branch and B1 from Beaver Dam Creek) are indicated with [a] if two-way ANOVA was used or [b] if t-test was used. Symbols are indicated next to concentrations that were the highest between the two streams.

Site	Component	Group	N	Ni	Cu	Zn	As	Se	Cd	Hg	Pb
Meyers Branch	CPOM	B1	3	8.38 ± 0.98	9.56 ± 0.98	46.05 ± 4.33	5.71 ± 1.23	0.69 ± 0.16	0.38 ± 0.07	0.09 ± 0.01	6.44 ± 1.35
	Benthic organic matter	B2	3	9.75 ± 1.10	8.51 ± 0.89	50.23 ± 4.83	5.10 ± 0.50	0.96 ± 0.10	0.41 ± 0.03	0.17 ± 0.03	18.19 ± 1.26
	Biofilm	B3	3	8.53 ± 5.08	19.13 ± 15.61	30.24 ± 9.38	1.93 ± 0.14	0.51 ± 0.05	0.94 ± 0.25	0.05 ± 0.01	12.06 ± 1.81
	Seston	B4	3	11.15 ± 0.28	10.27 ± 0.69	55.63 ± 2.03	7.78 ± 0.13	1.00 ± 0.03	0.41 ± 0.02	0.16 ± 0.01	19.99 ± 0.41
	Corbicula fluminea	C1	5	2.13 ± 0.22	73.41 ± 6.67	146.1 ± 11.17	3.92 ± 0.43	3.93 ± 0.43	2.65 ± 0.36	4.89 [b]± 0.72	1.83 ± 0.29
	Hydatophylax sp.	I1	5	1.20 ± 0.16	12.36 ± 0.89	162.9 [b]± 16.36	0.97 ± 0.29	0.28 ± 0.04	0.23 ± 0.04	0.02 ± 0.01	0.95 ± 0.15
	Gomphus sp.	I2	5	1.18 ± 0.22	21.41 ± 3.86	110.4 ± 8.29	1.55 ± 0.21	2.36 ± 0.31	0.19 ± 0.02	0.40 [b]± 0.08	3.43 ± 0.78
	Cambaridae	I3	5	0.50 ± 0.07	27.52 ± 1.73	55.98 ± 1.73	0.69 ± 0.07	1.41 ± 0.13	0.18 ± 0.04	0.11 [b]± 0.02	0.59 ± 0.15
	Anguilla rostrata	F1	5	1.12 ± 0.40	4.46 ± 1.91	57.88 ± 2.58	0.41 ± 0.05	2.37 ± 0.22	0.08 ± 0.01	1.00 [a]± 0.24	0.18 ± 0.03
	Semotilus atromaculatus	F2	5	0.88 ± 0.38	5.32 ± 1.38	94.71 ± 14.74	0.35 ± 0.08	1.68 ± 0.44	0.07 ± 0.01	0.48 [a]± 0.06	0.09 [a]± 0.03
	Notropis lutipinnis	F3	5	23.92 ± 7.78	4.62 ± 0.77	205.3 ± 10.99	0.20 ± 0.08	1.47 ± 0.11	0.04 ± 0.01	0.37 [a]± 0.02	0.45 ± 0.32
	Lepomis auritus		2	0.39 ± 0.23	1.11 ± 0.33	68.59 ± 12.97	0.44 ± 0.08	3.11 ± 0.45	0.03 ± 0.00	0.41 ± 0.06	0.10 ± 0.05
Beaver Dam Creek	CPOM	B1	3	91.05 ± 41.64	66.59 ± 29.00	125.60 ± 38.97	34.61 ± 14.63	6.13 ± 2.83	7.44 ± 3.57	0.29 ± 0.10	8.30 ± 0.54
	Benthic organic matter	B2	3	91.30 [b]± 0.64	246.81 [b]± 15.32	193.01 [b]± 5.36	128.62 [b]± 12.46	10.40 [b]± 0.77	9.71 [b]± 0.23	0.31 [b]± 0.02	19.56 ± 1.47
	Biofilm	B3	3	163.75 [b]± 8.18	192.69 [b]± 12.28	256.90 [b]± 13.50	139.71 [b]± 6.13	7.39 [b]± 0.45	14.64 [b]± 0.81	0.44 [b]± 0.03	16.78 ± 1.48
	Seston	B4	3	72.55 [a]± 5.98	163.71 [a]± 15.91	162.62 [b]± 17.70	58.13 [b]± 4.86	5.25 [a]± 0.49	11.45 [a]± 2.62	0.22 ± 0.04	17.23 ± 1.87
	Filamentous algae		2	64.29 ± 19.76	116.98 ± 53.51	171.32 ± 37.27	49.80 ± 15.56	8.97 ± 1.87	7.28 ± 2.98	0.18 ± 0.07	12.04 ± 6.85
	Corbicula fluminea	C1	5	8.40 [b]± 0.92	329.41 [b]± 87.56	247.02 [b]± 44.80	9.60 [b]± 0.42	20.79 [b]± 1.00	26.07 [b]± 2.54	0.06 ± 0.02	1.26 ± 0.16
	Macronema sp.	I1	5	3.12 [a]± 0.36	39.51 [b]± 0.72	109.54 ± 9.39	5.87 [b]± 0.39	18.22 [b]± 0.72	3.45 [b]± 0.50	0.16 [b]± 0.01	2.87 ± 1.09
	Gomphus sp.	I2	5	4.35 [a]± 0.84	45.30 [b]± 6.41	106.77 ± 8.65	25.93 [b]± 8.51	17.54 [b]± 0.97	1.21 [b]± 0.19	0.01 ± 0.01	1.90 ± 0.29
	Cambaridae	I3	5	2.27 [a]± 0.20	195.40 [b]± 7.27	76.26 [b]± 3.42	4.50 [b]± 0.31	13.47 [b]± 1.60	3.66 [b]± 0.30	0.02 ± 0.01	0.98 ± 0.64
	Anguilla rostrata	F1	5	2.89 [b]± 0.64	6.31 ± 0.74	71.80 ± 8.15	1.30 [b]± 0.11	20.00 [b]± 1.76	0.79 [a]± 0.10	0.24 ± 0.12	0.20 ± 0.04
	Lepomis auritus	F2	6	1.15 ± 0.13	3.12 ± 0.54	95.21 ± 7.86	1.87 [b]± 0.12	21.14 [b]± 1.48	0.62 [a]± 0.06	0.18 ± 0.05	BDL
	Gambusia holbrooki	F3	5	11.43 ± 4.16	8.91 [b]± 1.41	193.46 ± 3.55	2.47 [b]± 0.29	21.39 [b]± 1.12	0.68 [a]± 0.07	0.05 ± 0.01	0.07 ± 0.01

Table 4.4. Pearson's correlations (r) between trace element concentrations in invertebrates from the two study streams. Correlations (r) ≥ 0.36 are significant at $p < 0.05$. Bold type indicates r ≥ 0.50.

	Ni	**Cu**	**Zn**	**As**	**Se**	**Cd**	**Hg**	**Pb**
Ni	1.0000	-	-	-	-	-	-	-
Cu	0.4403	1.0000	-	-	-	-	-	-
Zn	0.3699	-0.3565	1.0000	-	-	-	-	-
As	**0.8787**	0.4360	0.1109	1.0000	-	-	-	-
Se	**0.7668**	**0.6648**	-0.0925	**0.8356**	1.0000	-	-	-
Cd	**0.6581**	**0.7720**	-0.0140	**0.5662**	**0.8239**	1.0000	-	-
Hg	-0.2274	-0.2703	0.0876	-0.3000	-0.1972	-0.2746	1.0000	-
Pb	0.2902	-0.1607	0.4071	0.2574	0.2108	0.0844	**0.5230**	1.0000

Table 4.5. Pearson's correlations (*r*) between trace element concentrations in fishes from the two study streams. Correlations (r) ≥ 0.35 are significant at $p < 0.05$. Bold type indicates r ≥ 0.50.

	Ni	**Cu**	**Zn**	**As**	**Se**	**Cd**	**Hg**	**Pb**
Ni	1.0000	-	-	-	-	-	-	-
Cu	0.4487	1.0000	-	-	-	-	-	-
Zn	**0.7852**	0.4182	1.0000	-	-	-	-	-
As	0.0923	0.3406	0.1704	1.0000	-	-	-	-
Se	0.0284	0.2445	0.0458	**0.9391**	1.0000	-	-	-
Cd	0.0786	0.3418	0.0418	**0.8849**	**0.9555**	1.0000	-	-
Hg	-0.2714	-0.2068	-0.3844	**-0.6066**	**-0.6230**	**-0.6349**	1.0000	-
Pb	0.1882	0.0787	0.1168	-0.4471	-0.3873	-0.3133	0.1668	1.0000

Table 4.6. Comparison of mean trace element concentrations (±1SE) in *Corbicula fluminea* from the Savannah River Site and the Chattahoochee River. [1]Chattahoochee River sites (from Chapter 2). N=number of individuals analyzed.

Site	Stream Type	N	Ni	Cu	Zn	As	Se	Cd	Hg	Pb
Meyers Branch	reference	5	2.13 ± 0.22	73.41 ± 6.67	146.09 ± 11.17	3.92 ± 0.43	3.93 ± 0.43	2.65 ± 0.36	4.89 ± 0.72	1.83 ± 0.29
Beaver Dam Creek	coal	5	8.40 ± 0.92	329.41 ± 87.56	247.02 ± 44.80	9.60 ± 0.42	20.79 ± 1.00	26.07 ± 2.54	0.06 ± 0.02	1.26 ± 0.16
Nancy Creek[1]	urban	9	0.87 ± 0.03	36.71 ± 1.31	303.28 ± 22.97	3.02 ± 0.16	3.26 ± 0.14	1.55 ± 0.08	0.13 ± 0.01	0.83 ± 0.18
Peachtree Creek[1]	urban	9	1.13 ± 0.08	63.62 ± 2.14	467.49 ± 31.66	2.71 ± 0.08	4.65 ± 0.11	4.08 ± 0.19	0.21 ± 0.01	1.14 ± 0.10
Sope Creek[1]	urban	9	1.24 ± 0.11	42.36 ± 1.56	296.07 ± 28.99	3.31 ± 0.11	4.78 ± 0.11	2.59 ± 0.13	0.21 ± 0.01	1.18 ± 0.08
Plant McDonough[1]	coal	9	1.30 ± 0.05	61.24 ± 3.34	509.52 ± 35.73	2.26 ± 0.10	4.53 ± 0.19	2.04 ± 0.23	0.12 ± 0.01	0.46 ± 0.04
Plant Wansley[1]	coal	9	1.85 ± 0.26	40.39 ± 1.93	543.96 ± 17.65	5.03 ± 0.17	9.80 ± 0.26	2.30 ± 0.09	0.13 ± 0.00	0.63 ± 0.05
Plant Yates[1]	coal	9	1.33 ± 0.09	87.73 ± 7.99	380.75 ± 27.67	3.50 ± 0.12	13.60 ± 0.92	3.61 ± 0.91	0.17 ± 0.01	0.65 ± 0.03
Flat Shoals Creek[1]	reference	9	2.38 ± 0.13	35.64 ± 1.42	245.85 ± 10.36	2.80 ± 0.12	4.63 ± 0.15	2.13 ± 0.12	0.56 ± 0.04	0.57 ± 0.17
Snake Creek[1]	reference	9	1.02 ± 0.07	37.71 ± 2.36	334.39 ± 23.46	3.17 ± 0.17	3.73 ± 0.20	2.64 ± 0.12	0.36 ± 0.06	0.64 ± 0.06

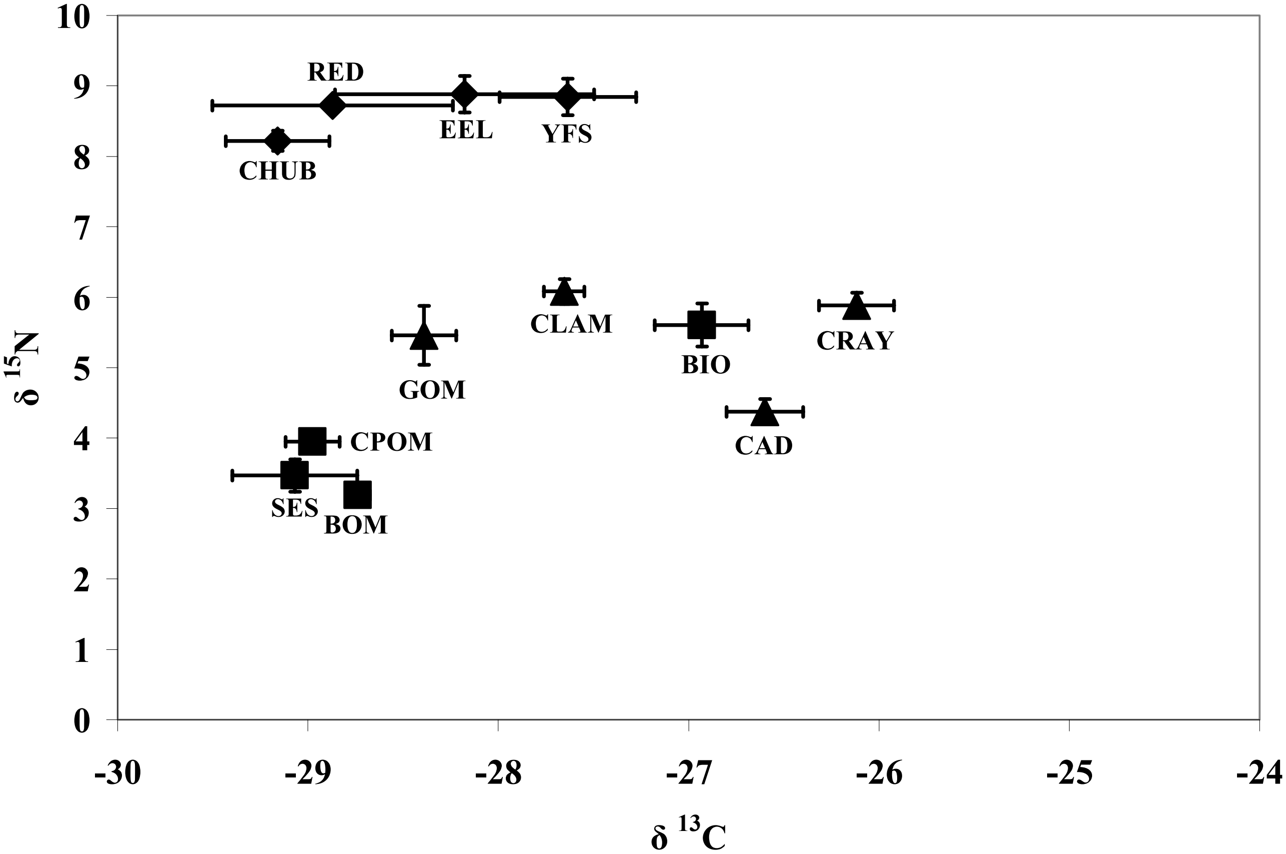
Figure Legend.

Figure 4.1. Relationship between stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) for food resources and organisms collected from Meyers Branch. Error bars indicate ±1SE. Closed diamonds=fishes, closed triangles=invertebrates, and closed squares=food resources. CHUB = creek chub, RED = redbreast sunfish, EEL = American eel, YFS = yellow fin shiner, GOM = dragonfly, CLAM = clam, CRAY = crayfish, CAD = caddisfly, BIO = biofilm, CPOM = coarse particulate organic matter, SES = seston, BOM = benthic organic matter.

Figure 4.2. Relationship between stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) for food resources and organisms collected from Beaver Dam Creek. Error bars indicate ±1SE. Closed diamonds=fishes, closed triangles=invertebrates, and closed squares=food resources. MOS = mosquitofish. All other abbreviations are described in Figure 4.1.

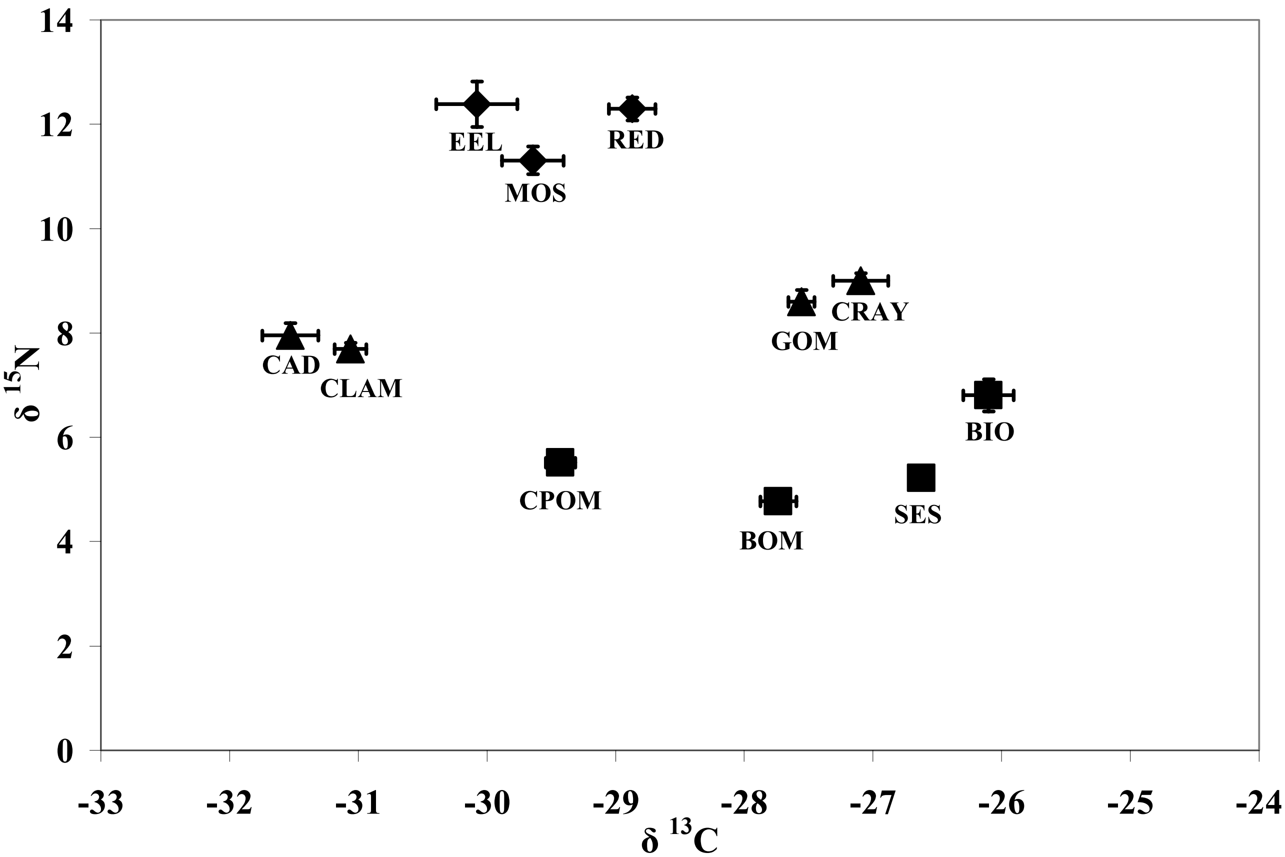
Figure 4.3. Log_{10} selenium concentrations in food web components from Beaver Dam Creek (closed diamonds) and Meyers Branch (open diamonds) plotted against $\delta^{15}N$ values. Regression equation for Meyers Branch: $y = 0.14 + 0.04x$, $r^2 = 0.21$, $p = 0.0008$. Regression equation for Beaver Dam Creek: $y = 0.67 + 0.06x$, $r^2 = 0.44$, $p < 0.0001$.

Figure 4.1.



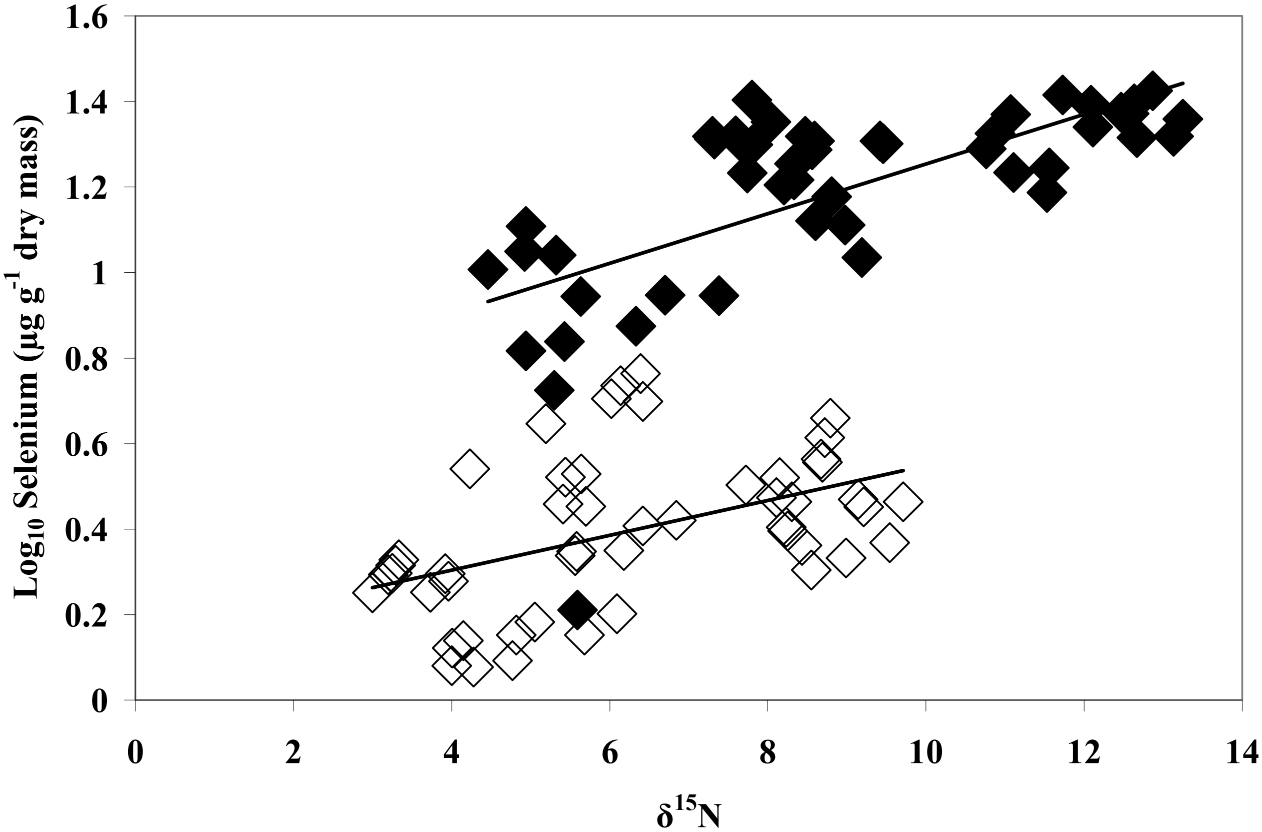
10
9
8
7
6
5
4
3
2
1
0
δ 15N
RED
EEL
YFS
CHUB
CLAM
CRAY
GOM
BIO
CAD
CPOM
SES
BOM
-30
-29
-28
-27
-26
-25
-24
δ 13C

Figure 4.2.



14
12
10
8
6
4
2
0
-33
-32
-31
-30
-29
-28
-27
-26
-25
-24
δ 15N
δ 13C
EEL
RED
MOS
CRAY
GOM
CAD
CLAM
BIO
CPOM
SES
BOM

Figure 4.3.


1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Log10 Selenium (µg g-1 dry mass)
0
2
4
6
8
10
12
14
δ15N

CHAPTER 5

POTENTIAL HUMAN HEALTH RISKS FROM CONSUMING FISH CAUGHT IN AN URBAN RIVER[1]

[1]Peltier, Gretchen Loeffler, Judy L. Meyer, Randy O. Manning, and Emma Rosi-Marshall. To be submitted to *Human and Ecological Risk Assessment.*

Abstract

Fish consumption guidelines have been established to provide protection for the health of recreational anglers. In the metropolitan Atlanta area, there are PCB and mercury fish consumption guidelines for carp, largemouth bass, and striped bass for sections of the Chattahoochee River. The primary objective of this study was to compare tissues concentrations of metals and metalloids in muscle fillets and livers from a common sportfish (*Lepomis macrochirus*) at four sites in the Chattahoochee River and relate observed concentrations to potential risks for human health using the results of angler surveys conducted by Upper Chattahoochee Riverkeeper. Concentrations of Ni, Cu, Zn, As, Se, and Cd in liver and muscle fillets were significantly different among the four study sites. Liver concentrations were consistently higher than muscle fillets for all trace elements except Pb and Hg. According to national fish guidance, consumption of fish should be limited based on arsenic (0.11-0.21 μg/g dry mass (DM)) and mercury (0.18-0.59 μg/g DM) concentrations in muscle fillets. Over half of African American and Hispanic anglers reported consuming fish from the river compared to only 26% of Caucasian anglers. Anglers consuming fish may be exposed to elevated concentrations of arsenic and mercury. These findings suggest that local community education and additional analyses of target species are warranted.

Introduction

Fish consumption guidelines have been established to provide protection for the health of recreational anglers. In 2004, the Environmental Protection Agency reported that 35% of the nation’s lake acres and 24% of river miles were under consumption advisories. Primary contaminants prompting fish advisories in the U.S. are mercury, PCBs, chlordane, dioxins, and

DDT (EPA 2004). Issuance of fish consumption advisories is the responsibility of the states, territories, and tribes, but federal agencies have issued advisories for larger regions (e.g. Great Lakes region). Communication of fish consumption advisories often does not reach all anglers, and the advisories often conflict with messages touting the dietary benefits of fish (Burger 2004, 2005). Anglers report informal communication (e.g., word-of-mouth) as the primary source of information about fish consumption advisories (Burger 2004).

In the metropolitan Atlanta area, fish consumption guidelines based on PCBs and mercury for carp, largemouth bass, and striped bass are in place for sections of the Chattahoochee River. During routine river patrols, Upper Chattahoochee Riverkeeper (UCR) staff observed anglers fishing reaches of the Chattahoochee River that were under fish consumption guidelines. UCR staffers were concerned that anglers were keeping their catch, suggesting that either the anglers were ignoring fish consumption guidelines or were unaware that these guidelines existed. In 1999, UCR staff began conducting surveys of anglers along the Chattahoochee River to quantify the behavior of anglers in the metropolitan Atlanta area (Loeffler et al. 2003).

In addition to PCBs and mercury, elevated concentrations of metals and metalloids have been observed in the Chattahoochee River. Previous studies have observed elevated concentrations of metals (e.g. copper, cadmium) and metalloids (e.g. arsenic and selenium), which we collectively term trace elements, in water (Froelich and Lesley 2001, Lesley and Froelich 2003), invertebrates (Rosi-Marshall 2002, Chapter 2), and fishes (Rosi-Marshall 2002) collected from the Chattahoochee River. The highest concentrations of arsenic and selenium in *Corbicula fluminea* (Chapter 2) and water (Froelich and Lesley 2001, Lesley and Froelich 2003) were approximately 10 - 20 km downstream of discharges from two coal-fired power plants.

The primary objective of the study was to compare trace element tissue concentrations in muscle fillets and livers from a widely distributed fish (*Lepomis macrochirus*) at four sites in the Chattahoochee River and relate observed concentrations to potential consumption risks for human health using the results of angler surveys (Loeffler et al. 2003) conducted by UCR. We predict that tissue concentrations in both muscle fillets and liver tissue will not differ among the study sites based on previous work in the Chattahoochee demonstrating no significant difference in trace element concentrations in muscle fillets of *Micropterus salmoides, Ictalurus punctatus*, and *Cyprinus carpio carpio* collected from the same sites (Rosi-Marshall 2002). We hypothesize that trace element concentrations in liver, an organ associated with metal metabolism, will be higher than concentrations in muscle fillets. We expect concentrations of trace elements associated with coal-fired power plant discharges (e.g., arsenic and selenium) to be correlated in liver and muscle tissues because three plants discharge into in the river.

Methods

Site description

Fishes were collected with a boat shocker in summer 1999 at four sites along the mainstem of the Chattahoochee River (Morgan Falls, Atlanta Road, Highway 166, and Franklin) and frozen for storage. Morgan Falls was the furthest upstream site and was located downstream of a hydroelectric dam. The Atlanta Road site was located where Atlanta Road crosses the Chattahoochee River downstream of a large tributary with an urbanized catchment. Hwy 166 was downstream of most industrial and municipal sources in the metropolitan Atlanta area. The Franklin site was located at the Georgia Hwy 27 crossing, which is approximately 160 km downstream of Atlanta and 20 km downstream of two coal-fired power plant discharges. Many anglers utilize this location to fish from the bank or launch their boats.

Fish tissues

Bluegill (*Lepomis macrochirus*) were selected as the fish to analyze because they are widely distributed, consumed by anglers, and because we were able to collect them at a frequency (N=5 per site) suitable for comparisons among the four study sites. After thawing fish, their total length and wet weight was measured, and muscle fillets and livers were removed, frozen, freeze-dried, and homogenized. Total length of all fish analyzed was similar (125 ± 7 mm). We chose to analyze muscle fillets since they are the most common part of the fish consumed by anglers. Livers were analyzed to determine if higher concentrations of trace elements were present, which could pose a potential health risk to individuals consuming the entire fish. In addition, liver tissues have been used to infer common contaminant sources because fishes exposed to similar contaminant sources exhibit correlations among trace elements in liver tissue (Kirby et al. 2001).

Trace element analysis

Approximately 20-60 mg dry mass (DM) of livers and 100 mg DM of muscle tissues were used for digestion. Trace metal grade nitric acid (2.5 ml for livers and 5 ml for muscle tissues) was added to the samples prior to digestion in a microwave (CEM Corporation, Matthews, NC, USA) with heating steps of 60, 60, 70, 80% microwave power for 10, 10, 15, and 20 minutes, respectively. After digestion, 0.5 ml of trace metal grade hydrogen peroxide was added, and the samples were microwaved for the same duration as the nitric acid digestion. Samples were brought to a final volume of 10 ml with deionized-distilled water. All trace element analyses were performed using an inductively coupled plasma mass spectrometer (ICP-

MS) (Perkin Elmer, Norwalk, CT). We measured total concentrations of vanadium (V), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), cadmium (Cd), mercury (Hg), and lead (Pb). Concentrations of V, Mn, Fe, Co, and Sr are not discussed further, but all of the trace element concentrations for tissues are reported in Appendix D. Certified reference material (DORM, DOLT, Tort 2; NRC, Ottawa, Canada) and blanks were included in the digestion and analysis procedure. Mean percent recoveries ranged from 89 – 115%. Tissue concentrations were expressed as μg/g dry mass (DM).

Angler surveys

During water quality monitoring trips and routine river patrols between 1999 and 2002, UCR staff interviewed 112 anglers who were fishing either from the bank or in boats along the Chattahoochee River between Morgan Falls and Franklin, Georgia. Interviews took place primarily during the week and throughout the daylight hours. Interviewers identified themselves as UCR staff and inquired if the anglers would be willing to participate in a survey regarding fishing behavior. Anglers were asked a series of nine questions that addressed the frequency of fishing and fish consumption, types of fish caught, and whether or not fish were shared with others (Table 5.1). The interviewers also noted the age, location, and ethnicity of the angler. Bluegill and other sunfishes were included in the bream category on the survey.

Statistical analyses

We tested the normality of trace element concentrations with the Shapiro-Wilk's test and transformed them using $\log_{10}(x+1)$. One-way ANOVAs were used to determine if trace element concentrations were significantly different among the study sites in liver and muscle fillets followed by Tukey-Kramer post hoc tests. We also used one-way ANOVAs to compare Cu, As, Cd, and Hg concentrations in bluegill muscle fillets with bass and catfish fillets collected concurrently (Rosi-Marshall 2002). We conducted paired t-tests to compare muscle and liver tissue at each site. Pearson's correlation coefficient (*r*) was used to explore relationships among trace element concentrations in muscle fillets and livers to look for evidence of common sources of contaminants. JMP 5.0.1 (SAS Institute, Cary, NC, USA) was used for statistical analyses.

Results

Trace element concentrations

Concentrations of Ni, Cu, Zn, Cd, Hg, and Pb in muscle fillets were not significantly different among the four study sites (ANOVA, $p > 0.05$, Table 5.2). As and Se concentrations in muscle fillets were significantly higher (ANOVA, $p < 0.05$) in bluegill from the Franklin site (Table 5.2). In liver tissue, As was the only trace element that was significantly different (ANOVA, $p < 0.05$) among the four study sites, and it was highest in bluegill from the Franklin site (Table 5.2).

Concentrations of Ni, Cu, Zn, As, Se, and Cd were significantly different in liver and muscle fillets (paired t-test, $p < 0.05$) (Tables 5.2 and 5.3). Liver concentrations were consistently higher than muscle fillets for all trace elements except Pb and Hg (Table 5.2 and 5.3). Zn, As, and Se concentrations in liver were approximately twice as high as concentrations in muscle fillets whereas Cu concentrations differed by an order of magnitude (Table 5.2).

Correlations among trace elements in liver tissue have been attributed to similar sources of exposure (Kirby et al. 2001). In liver tissue, four pairs of trace elements were significantly correlated: Ni and Se ($r = -0.51$, $p = 0.045$), Ni and Hg ($r = 0.67$, $p = 0.004$), Se and Cd ($r = 0.64$, $p = 0.008$), and Hg and Pb ($r = 0.59$, $p = 0.015$). However, As and Se were the only trace elements significantly correlated in bluegill muscle fillets ($r = 0.66$, $p = 0.002$). As and Se were not significantly correlated in liver tissue.

Angler surveys

Preliminary results from the angler surveys were reported in Loeffler et al. (2003). In total 112 anglers were interviewed, and 46% of anglers reported that they consumed fish from the river (Table 5.3). Fifty-one percent of anglers were African American, 38% Caucasian, 9% Hispanic, and 1% Asian. Fifty-five percent of African American and 89% of Hispanic anglers reported that they consumed fish on a regular basis compared to 26% of Caucasian anglers (Table 5.3). Each ethnic group reported differences in the type of fish caught and eaten. African American and Hispanic anglers commonly consumed carp and catfish whereas Caucasian anglers reported consuming equal percentages of bass, catfish, and carp (Table 5.4). Consumption of bream (e.g., bluegill) ranged from 6 - 23% of anglers (Table 5.4).

Discussion

Site differences

Trace element concentrations in bluegill muscle and liver tissue did not differ greatly among the four study sites. As and Se were the only two elements that differed significantly among sites, and the Franklin site was significantly higher for both (Table 5.2). A likely source of As and Se is discharges from coal-fired power plants, which are approximately 20 km upstream from Franklin, but downstream of all other sites. Several studies have shown elevated dissolved and particulate trace element concentrations in water (Froelich and Lesley 2001, Lesley 2002, Lesley and Froelich 2003) and in tissues of an indicator organism (Chapter 2) downstream of coal-fired power plant discharges in this river. However, bass muscle fillets collected at the same time from the Franklin site did not have elevated As or Se concentrations (Table 5.5). Bluegill and bass differ in feeding strategies and mobility, which may explain the differences observed. Bass are highly mobile predators whereas bluegill are more sedentary generalist feeders (Burger et al. 2001a). Bluegill primarily feed on particles suspended in the water column. Elevated As and Se concentrations in bluegill muscle fillets may be in response to elevated particulate concentrations of As and Se observed in the 10 – 20 km reach of the river downstream of coal-fired power plant discharges (Froelich and Lesley 2001, Lesley 2002, Lesley and Froelich 2003).

When mean concentrations of Cu, As, and Hg in muscle fillets were compared among three species of fish (bluegill, catfish, and bass), no significant differences in Cu and Hg were observed among species at any of the study sites (Table 5.5). However, bluegill had significantly lower concentrations (ANOVA, $p<0.05$) of As than bass and catfish. Bass and catfish feed at a higher trophic level than bluegill (Burger et al. 2001a), which may explain the differences observed for As (Table 5.5). When fish consumption advisories are issued, warnings usually focus on higher trophic level fishes (e.g. bass), which is appropriate since interviewed anglers preferred consuming higher trophic level species (Table 5.4). Cu and Hg did not differ among species.

Trace element concentration differences in liver vs. muscle tissue

To compare concentrations observed in this study to wet mass (WM) concentrations reported in the literature, we assumed 75% moisture content when converting concentrations expressed per g WM to g DM (Hamilton 2004). Se concentrations in bluegill muscle fillets ranged from 2.5 to 4.0 µg/g DM across the four study sites (Table 5.2). In comparison, Se concentrations in largemouth bass muscle fillets exposed to coal fly ash discharging into Rogers

Quarry, Tennessee were 12 µg/g DM (3 µg/g WM). In 1989, fly ash disposal in the system was phased out and Se concentrations in largemouth bass muscle fillets decreased to 4 µg/g DM (1 µg/g WM) six years following elimination of coal ash discharges (Southworth et al. 1994, Southworth et al. 2000). In largemouth bass from another coal site, the D-area ash basin system at the Savannah River Site (SRS), Se concentrations were 12 µg/g DM (3 µg/g WM) and 0.72 µg/g DM (0.18 µg/g WM) at a reference site (Jackson et al. 2002). As concentrations in bluegill muscle fillets in the Chattahoochee River ranged from 0.11 to 0.21 µg/g DM (Table 5.2). For comparison, mean concentrations of As in muscle fillets of largemouth bass from a contaminated SRS site were 0.32 µg/g DM (0.08 µg/g WM) and 0.04 µg/g DM (0.01 µg/g WM) at a reference site (Jackson et al. 2002). Thus, concentrations of As and Se in bluegill muscle fillets in the Chattahoochee River are lower than concentrations in muscle fillets of largemouth bass exposed to coal ash discharges in Tennessee and South Carolina. However, As and Se concentrations in bluegill in the Chattahoochee River were higher than concentrations found in largemouth bass from the reference site (Fire Pond) used in the South Carolina study (Jackson et al. 2002).

Liver concentrations were consistently higher than concentrations in muscle fillets, which has been observed in other studies (Mason et al. 2000, Kirby et al. 2001, Jackson et al. 2002). However, no significant differences were found in muscle vs. liver concentrations of Hg and Pb (Table 5.2), which has been shown previously for Hg (Amundsen et al. 1997, Viana et al. 2005). Liver concentrations of Cu, Zn, As, and Cd in this study were comparable to liver concentrations in bluegill from Ohio streams receiving discharges from coal-fired power plants (Table 5.6) (Lohner et al. 2001). Liver may be a more sensitive measure of exposure, but from the standpoint of human health, concentrations in muscle fillets are more relevant.

Fishing behavior

We observed differences in angler behavior based on ethnicity (Tables 5.3 and 5.4) with minority (Asian, Hispanic, African American) anglers reporting more consumption than Caucasian anglers. Using relative risk calculations, minority anglers were twice as likely to consume fish from the river than non-minority anglers (Loeffler et al. 2003). Other studies have also shown significant relationships between ethnicity and consumption behavior (Burger et al. 1999, Burger et al. 2001b, Burger 2004, Moya 2004). Our conclusions for total amount of fish consumed were limited since the survey questions addressed frequency not actual amounts of fish consumed. However, we were able to observe differences in frequency, which is valuable for understanding behavior and potential health risks to populations.

Comparison to human health standards

We compared trace element concentrations in bluegill muscle fillets to risk-based concentration tables (µg/g WM) from EPA region III. These concentrations were calculated using fish consumption of approximately 11 oz of fish/week over a 30 year period. Ni, Cu, Zn, Se, and Cd concentrations in bluegill muscle fillets were below the risk-based concentration levels. However, As concentrations ranging from 0.11-0.21 µg/g DM at all four sites far exceed the recommended limit of 0.0084 µg/g DM (0.0021 µg/g WM). Hg concentrations in bluegill (0.59 µg/g DM) from Morgan Falls exceeded the standard of 0.56 µg/g DM (0.14 µg/g WM). We also compared As and Hg concentrations to EPA's national fish consumption guidance, which is calculated for adults (males and non-pregnant females). Based on As concentrations in bluegill muscle, adults should consume no more than two 8 oz meals/month. For mercury, recommended consumption of bluegill should not exceed four 8 oz meals/month (EPA 2000). These consumption standards are conservative measures, but they suggest that consumption of bluegill and other fishes should be limited in this river.

Human health standards for arsenic are based on concentrations of inorganic arsenic and associated compounds. Organic forms of arsenic (e.g., arsenobetaine) are not included since they do not threaten human health (EPA 2000). We measured total arsenic concentrations in muscle and liver tissue, which includes inorganic and organic forms of arsenic. Concentrations of inorganic arsenic ranged from 15-30% of total arsenic in freshwater fish and 10-25% of total arsenic in marine fishes (Lawrence et al. 1986). Though a uniform standard for percent inorganic or organic arsenic in freshwater or marine fishes for human health assessments does not currently exist, EPA Region VI uses 30% inorganic arsenic in their calculations for human health recommendations. Using this standard, calculated inorganic concentrations in bluegill muscle fillets (0.033 - 0.063 μg/g DM) still exceed the recommended limit of 0.0084 μg/g DM. Many laboratories can only measure total trace element concentrations and do not have the ability to differentiate among the different species (e.g. arsenobetaine). Therefore, further analysis describing the organic/inorganic portioning of arsenic in freshwater and marine fishes is needed, and this information can then be incorporated into uniform standards.

Conclusion

Tissue (liver and muscle fillet) concentrations of all trace elements (except As and Se) in bluegill did not significantly differ among the four study sites in the Chattahoochee River. As and Se concentrations were significantly higher in bluegill from the Franklin site, likely a result of discharges from upstream coal-fired power plants. As expected, liver concentrations were consistently higher than muscle fillets for all trace elements except Hg and Pb. As concentrations in muscle fillets at all sites suggest the need for at least some restrictions on consumption. Differences in fish consumption patterns and behavior were related to ethnic differences of the anglers, although all reported greater consumption of higher trophic level fishes (e.g., bass). Anglers consuming bluegill from the Chattahoochee River may be exposed to elevated concentrations of arsenic.

Acknowledgements

We would like to thank Heather A. Brant, Brian P. Jackson, William A. Hopkins, and the Advanced Analytical Center for Environmental Sciences at the Savannah River Ecology Laboratory for assistance in ICP-MS analysis. We would also like to acknowledge the countless hours that Riverkeeper staff (especially Harlan Trammel) and volunteers devoted to collecting survey information from local anglers. The Waterfall Foundation, Turner Foundation, and the Price Gilbert, Jr. Charitable Foundation funded the development, collection and analysis of angler surveys.

Literature Cited

Amundsen, P. A., F. J. Staldvik, A. A. Lukin, N. A. Kashulin, O. A. Popova, and Y. S. Reshetnikov. 1997. Heavy metal contamination in freshwater fish from the border region between Norway and Russia. Science of the Total Environment **201**:211-224.

Burger, J. 2004. Fish consumption advisories: knowledge, compliance and why people fish in an urban estuary. Journal of Risk Research **7**:463-479.

Burger, J. 2005. Fishing, fish consumption, and knowledge about advisories in college students and others in central New Jersey. Environmental Research **98**:268-275.

Burger, J., K. F. Gaines, C. S. Boring, W. L. Stephens, J. Snodgrass, and M. Gochfeld. 2001a. Mercury and selenium in fish from the Savannah River: Species, trophic level, and locational differences. Environmental Research **87**:108-118.

Burger, J., K. F. Gaines, and M. Gochfeld. 2001b. Ethnic differences in risk from mercury among Savannah River fishermen. Risk Analysis **21**:533-544.

Burger, J., W. L. Stephens, C. S. Boring, M. Kuklinski, J. W. Gibbons, and M. Gochfeld. 1999. Factors in exposure assessment: Ethnic and socioeconomic differences in fishing and consumption of fish caught along the Savannah River. Risk Analysis **19**:427-438.

EPA. 2000. Guidance for assessing chemical contaminant data in fish advisories. EPA 823-B-00-008.

EPA. 2004. National Listing of Fish Advisories. Cincinnati, OH.

Froelich, P. N., and P. Lesley. 2001. Tracing germanium contamination from coal-fired power plants along the Chattahoochee River-Apalachicola River: implications for the toxic metalloids arsenic and selenium. *in* K. J. Hatcher, editor. Proceedings of the Georgia Water Resources Conference. Institute of Ecology, University of Georgia, Athens, GA.

Hamilton, S. J. 2004. Review of selenium toxicity in the aquatic food chain. Science of the Total Environment **326**:1-31.

Jackson, B. P., P. L. S. Allen, W. A. Hopkins, and P. M. Bertsch. 2002. Trace element speciation in largemouth bass (Micropterus salmoides) from a fly ash settling basin by liquid chromatography-ICP-MS. Analytical and Bioanalytical Chemistry **374**:203-211.

Kirby, J., W. Maher, and D. Harasti. 2001. Changes in selenium, copper, cadmium, and zinc concentrations in mullet (Mugil cephalus) from the southern basin of Lake Macquarie, Australia, in response to alteration of coal-fired power station fly ash handling procedures. Archives of Environmental Contamination and Toxicology **41**:171-181.

Lawrence, J. F., P. Michalik, G. Tam, and H. B. S. Conacher. 1986. Identification of arsenobetaine and arsenocholine in Canadian fish and shellfish by high-performance liquid-chromatography with atomic-absorption detection and confirmation by fast-atom-bombardment mass-spectrometry. Journal of Agricultural and Food Chemistry **34**:315-319.

Lesley, P. 2002. The fluxes and fates and arsenic, selenium, and antimony from coal fired power plants to rivers. Masters Thesis. The Georgia Institute of Technology, Atlanta.

Lesley, P., and P. N. Froelich. 2003. Arsenic, selenium, and antimony: from coal fired power plants to the Chattahoochee River. *in* K. J. Hatcher, editor. Proceedings of the 2003 Georgia Water Resources Conference. University of Georgia, Athens, GA.

Loeffler, G., J. L. Meyer, H. Trammell, and S. Holmbeck-Pelham. 2003. Fish consumption patterns along the Upper Chattahoochee River. *in* K. J. Hatcher, editor. Proceedings of the 2003 Georgia Water Resources Conference. University of Georgia, Athens, GA.

Lohner, T. W., R. J. Reash, V. E. Willet, and L. A. Rose. 2001. Assessment of tolerant sunfish populations (Lepomis sp.) inhabiting selenium-laden coal ash effluents - 1. Hematological and population level assessment. Ecotoxicology and Environmental Safety **50**:203-216.

Mason, R. P., J. M. Laporte, and S. Andres. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. Archives of Environmental Contamination and Toxicology **38**:283-297.

Moya, J. 2004. Overview of fish consumption rates in the United States. Human and Ecological Risk Assessment **10**:1195-1211.

Rosi-Marshall, E. J. 2002. Quality of suspended fine particulate matter and its role as a conduit for metals in riverine food webs. Ph.D. The University of Georgia, Athens.

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

Southworth, G. R., M. J. Peterson, and R. R. Turner. 1994. Changes in concentrations of selenium and mercury in largemouth bass following elimination of fly-ash discharge to a quarry. Chemosphere **29**:71-79.

Viana, F., R. Huertas, and E. Danulat. 2005. Heavy metal levels in fish from coastal waters of Uruguay. Archives of Environmental Contamination and Toxicology **48**:530-537.

Table 5.1. Fish consumption survey questions.

Question	**Response Categories**
Have you been interviewed by Riverkeeper before?	Yes/no
How often do you fish here?	Every day, 2-3/wk, 1/wk, 2-3/mn, 1/mn, <1/mn
What kind of fish do you usually catch here?	Trout, crappie, catfish, striper, bass, carp, bream, other
How large are the fish that you usually catch here?	<8", 8-12", >12"
Do you eat the fish that you catch?	Always, sometimes, never, used to but not anymore
If you eat the fish, what kind do you usually eat?	Trout, crappie, catfish, striper, bass, carp, bream, other
How often do you eat the fish?	Every day, 2-3/wk, 1/wk, 2-3/mn, 1/mn, <1/mn
Do you share the fish you catch with others who eat them?	Always, sometimes, never
Have you ever caught fish here that were unusual in appearance?	Yes/no

Table 5.2. Mean concentrations (±1SE) of trace elements in liver and muscle fillets of bluegill. All concentrations are µg/g dry mass.

Site	**Tissue**	**N**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Cd**	**Hg**	**Pb**
Morgan Falls	fillet	5	0.05 ± 0.02	0.72 ± 0.08	48.95 ± 10.45	0.11 ± 0.02	2.58 ± 0.12	BDL	0.59 ± 0.26	0.21 ± 0.23
Morgan Falls	liver	5	0.32 ± 0.07	11.74 ± 2.42	106.48 ± 7.76	0.59 ± 0.04	8.04 ± 0.46	1.23 ± 0.47	0.42 ± 0.06	0.06 ± 0.04
Atlanta Road	fillet	5	0.14 ± 0.02	1.29 ± 0.30	45.01 ± 4.01	0.16 ± 0.02	2.81 ± 0.43	BDL	0.29 ± 0.05	BDL
Atlanta Road	liver	2	0.68 ± 0.45	4.66 ± 1.92	108.28 ± 0.85	0.45 ± 0.11	4.82 ± 0.40	0.18 ± 0.12	0.25 ± 0.04	0.21 ± 0.23
Hwy166	fillet	5	0.09 ± 0.03	0.74 ± 0.03	33.12 ± 2.05	0.12 ± 0.01	3.57 ± 0.24	BDL	0.24 ± 0.03	BDL
Hwy166	liver	3	0.33 ± 0.16	7.44 ± 1.06	104.04 ± 22.74	0.49 ± 0.18	7.70 ± 0.73	0.96 ± 0.28	0.46 ± 0.14	0.17 ± 0.12
Franklin	fillet	5	0.08 ± 0.03	0.85 ± 0.23	36.77 ± 3.28	0.21 ± 0.03	4.04 ± 0.32	BDL	0.18 ± 0.03	BDL
Franklin	liver	6	0.50 ± 0.21	8.52 ± 1.02	120.34 ± 12.67	1.32 ± 0.22	7.35 ± 0.66	0.84 ± 0.21	0.54 ± 0.20	0.12 ± 0.09

Table 5.3. Summary of fish consumption frequency grouped by ethnicity of angler.

Ethnicity	Never	<1/month	1/month	2-3/month	1/week	2-3/week	Total Number	% Eating fish
African American	24	6	8	3	11	1	53	55
Asian	1	0	0	1	0	0	2	50
Caucasian	29	2	4	2	2	0	39	26
Hispanic	1	1	1	3	2	1	9	89

Table 5.4. Percentages of fish consumed by species from the Chattahoochee River grouped by angler ethnicity.

	Bass	**Bream**	**Carp/Catfish**	**Crappie**	**Trout**
African American	20%	23%	38%	16%	4%
Asian	0%	100%	0%	0%	0%
Caucasian	28%	6%	28%	17%	22%
Hispanic	17%	11%	61%	6%	6%

Table 5.5. Comparison of mean trace element concentrations (±1SE) in muscle fillets from three fish taxa collected from the Chattahoochee River. Tissue concentrations are µg/g dry mass. NA=no samples analyzed, BDL=below detection limit, and no standard errors given for samples where N=1. [a]From Rosi-Marshall (2002).

Site	Taxa	Cu	As	Cd	Hg
Morgan Falls	Bluegill	0.72 ± 0.08	0.11 ± 0.02	BDL	0.59 ± 0.26
	Bass[a]	0.73 ± 0.09	4.54 ± 0.28	0.01 ± 0.001	1.59 ± 0.65
	Catfish[a]	0.80	4.31	0.01	0.62
Atlanta Road	Bluegill	1.29 ± 0.30	0.16 ± 0.02	BDL	0.29 ± 0.05
	Bass[a]	0.90	2.94	0.01	0.38
	Catfish[a]	1.10	4.20	0.01	0.48
Hwy166	Bluegill	0.74 ± 0.03	0.12 ± 0.01	BDL	0.24 ± 0.03
	Bass[a]	0.80	4.26	0.01	0.88 ± 0.29
	Catfish[a]	0.90	6.11	0.02	0.33
Franklin	Bluegill	0.85 ± 0.23	0.21 ± 0.03	BDL	0.18 ± 0.03
	Bass[a]	1.00 ± 0.11	3.44 ± 0.45	0.01 ± 0.002	1.15
	Catfish[a]	NA	NA	NA	NA

Table 5.6. Liver tissue trace element concentrations (mean ± 1SD) in bluegill from this study and three reference and contaminated streams in Ohio (Lohner et al. 2001) expressed in µg/g dry mass. [a]From Lohner et al. 2001.

Site	Cu	Zn	As	Se	Cd	Pb
Morgan Falls	11.74 ± 5.41	106.48 ± 17.36	0.59 ± 0.09	8.04 ± 1.03	1.23 ± 1.05	0.06 ± 0.09
Atlanta Road	4.66 ± 2.71	108.28 ± 1.20	0.45 ± 0.15	4.82 ± 0.56	0.18 ± 0.17	0.21 ± 0.32
Hwy166	7.44 ± 1.83	104.04 ± 39.38	0.49 ± 0.31	7.70 ± 1.26	0.96 ± 0.48	0.17 ± 0.21
Franklin	8.52 ± 2.50	120.34 ± 31.03	1.32 ± 0.54	7.35 ± 1.62	0.84 ± 0.52	0.12 ± 0.22
Campaign Creek[a]	6.70 ± 0.40	86.10 ± 7.30	0.64 ± 0.40	2.80 ± 1.80	0.70 ± 0.20	2.20 ± 3.30
Ohio River[a]	12.10 ± 6.10	90.00 ± 23.40	1.70 ± 1.20	8.20 ± 4.20	0.90 ± 0.50	0.40 ± 0.40
Stingy Run[a]	4.60 ± 1.50	93.80 ± 11.70	1.70 ± 0.50	24.70 ± 9.10	0.80 ± 0.60	11.50 ± 15.00

CHAPTER 6

CONCLUSIONS

Most trace element research with aquatic organisms has focused on changes in community assemblages downstream of mining activities in lotic systems (e.g., Clements 1994, Clements and Kiffney 1995, Farag et al. 1998, Beltman et al. 1999, Clements et al. 2000) and community changes and accumulation patterns in lentic systems (e.g., Guthrie and Cherry 1979, Lemly 1985, 1997, Crutchfield 2000, Hopkins et al. 2000, Rowe et al. 2001, Jackson et al. 2002, Lemly 2002, Pedlar et al. 2002, Rowe et al. 2002, Hopkins et al. 2004b). Much less is known about accumulation of trace elements in lotic food webs (e.g., Mason et al. 2000, Besser et al. 2001, Lohner et al. 2001b, Simmons and Wallschlager 2005). In this dissertation, I addressed trace element accumulation patterns in a stream-dwelling indicator organism (*Corbicula fluminea*), in *Lepomis macrochirus,* and in two lotic food webs.

In Chapter 2, I asked if trace element concentrations in *Corbicula fluminea* tissue were associated with particular point and nonpoint sources of trace element contamination in an urban river. Two point source types (wastewater and coal-fired power plants (CFPPs)) and three nonpoint source types (forest, agriculture, and urban) were sampled. Among the fifteen study sites, clams from nine sites had trace element concentrations that were significantly higher or lower than the other locations. Concentrations in clams from the remaining six sites were consistently in the middle of the range observed in the study. Clams from forested catchments had significantly higher tissue concentrations of mercury. Clams from sites near CFPP discharges had higher tissue concentrations of arsenic, selenium, and cadmium. Clams from sites with highly urbanized catchments had higher concentration of cadmium and zinc. Non-metric multidimensional scaling (NMS) based on trace element concentrations in clams grouped clams from some of the agriculture and forest sites together whereas clams from one urban stream and sites near CFPP discharges clustered at the opposite end of the ordination space. CFPP discharges are a point source of trace element contamination in this urban river.

Having identified CFPPs as sources of trace element contamination in an urban river (Chapter 2), I further explored the temporal patterns of trace element accumulation and consequences for growth in *Corbicula fluminea* (Chapter 3). I transplanted clams from a reference stream to a stream receiving input from only one point source, discharge from the ash pond of a CFPP. Concentrations of nickel, arsenic, selenium, and cadmium were significantly higher in clams from the stream site closest to the CFPP discharge (Site A). Mercury concentrations in transplanted clams at Site A declined over time and were significantly lower than at all other sites after three months. The selenium-rich environment at Site A contributed to the decline in mercury concentrations because clams from downstream sites with lower selenium concentrations did not exhibit the rapid decline in mercury concentrations. Despite elevated trace element content of tissues, clams from Site A had the highest growth rates, a consequence of warmer temperatures at that site. Glutathione (a biomarker) concentrations were significantly higher at the most contaminated site (A) which suggests induction of detoxifying mechanisms at the cellular level. In summary, despite the fact that clams accumulated high concentrations of trace elements downstream of a CFPP discharge, their growth rates were not affected, but they exhibited a response at the cellular level. Other factors (e.g., temperature and perhaps food availability) diminished the impacts of living in a contaminated environment.

In the two chapters just discussed, I observed accumulation of trace elements in *Corbicula fluminea* downstream of CFPP discharges. In Chapter 4, I asked whether elevated concentrations in clams were an indicator of more widespread contamination of the food web. I sampled representative components of the food web in two streams, one reference and one contaminated with CFPP discharge. Clams from the contaminated stream had significantly higher concentrations of nickel, copper, zinc, arsenic, selenium, and cadmium than clams from the reference stream. Similarly, food resources, invertebrates, and fishes from the contaminated stream had significantly higher concentrations of trace elements than comparable food web components from the reference stream. Mercury concentrations in clams and the rest of the food web of the reference stream were consistently higher than in the contaminated stream. Selenium biomagnified from basal resources to generalist fishes in both streams. Clams appear to be a reliable indicator of food web contamination in highly contaminated streams, but their utility may be limited in less impacted, reference streams.

Chapter 5 focuses on the potential human health risks from consumption of fishes from an urban river through the analysis of angler survey and trace element concentrations in bluegill (*Lepomis macrochirus)*, a common fish, in the Chattahoochee River. Only arsenic and selenium concentrations in bluegill tissue significantly differed among four study sites along the river. Trace element concentrations were significantly higher in liver tissue compared to muscle fillets. Arsenic concentrations exceeded consumption guidelines from EPA region III. Angler surveys indicated differences in fishing and consumption behavior among ethnic groups. Minority anglers were twice as likely to consume fish from the river. Anglers consuming fishes from the river may be exposed to elevated concentrations of arsenic. This is important because exposure to high concentrations of arsenic through diet represents a significant public health risk.

Community education and further analysis of trace element content of fishes in the Chattahoochee River is warranted to further assess the risks to human health.

This research reinforces the notion that aquatic discharges into streams and rivers from the ash-settling basins of CFPPs should not be viewed as a benign alternative to landfills or minefills. These discharges can lead to uptake of trace elements by aquatic organisms and subsequent transfer through the food web. In larger rivers, the spatial extent of contamination may be smaller because of dilution, but hotspots of invertebrates with high body burdens result in transfer of these contaminants to higher trophic levels. In streams receiving CFPP discharges, arsenic and selenium concentrations in aquatic organisms were elevated, and evidence for selenium biomagnification was observed (Chapters 2-4). Concentrations of selenium in clams from the Chattahoochee River and invertebrates in South Carolina exceed the proposed dietary threshold of 3 μg/g dry mass for fish (Lemly 1993b, Hamilton 2002, 2004). Fish consuming clams from these rivers would likely have reduced growth and survival.

Alternative uses of coal combustion wastes have been developed in lieu of disposal in surface impoundments, minefills, and landfills. The utilization of alternatives has increased from 25% in 1995 to 38% in 2003 as the demand for coal combustion wastes increases (NRC 2006). Fly ash is used to replace raw materials such as silica and calcium in the production of cement and contributes to the long-term strength of the cement product. Fly and bottom ash is also used in a variety of engineered fill projects including road base materials and embankments. Coal combustion wastes are used as synthetic gypsum in wallboard manufacturing and using synthetic gypsum minimizes the consumption of virgin materials. Many other applications exist including soil amendments, roofing shingles, and abrasives for sandblasting. In 2000, EPA determined that these alternative uses are not likely to present significant risks to human health or the environment (NRC 2006). The long-term health and environmental effects are not fully understood, but these alternatives could minimize the volume of coal combustion wastes disposed into surface impoundments and subsequent release of trace elements into neighboring rivers and streams.

Corbicula fluminea is a useful and simple tool for assessing food web contamination because of ease of collection and tissue analysis that is less costly than analysis of multiple samples of food resources, invertebrates, and fishes in a river. In addition, *Corbicula* is an introduced species so the ethical dilemmas and permitting constraints associated with native fauna are minimized. Trace element contamination detected with clams and resulting from discharges to an urban river from CFPPs and other sources has the potential to impact populations of anglers. Minority populations seem most vulnerable. Consumption of fishes from rivers receiving CFPP discharges may result in increased exposure to trace elements (e.g., arsenic and mercury), which are correlated with increased disease risk from chronic exposure.

This dissertation focused on trace element accumulation patterns in lotic food webs because less is known about those patterns in flowing waters, which are typically thought to tolerate greater loading of trace elements than lakes and reservoirs. Bioaccumulation of trace elements in both target organisms and food webs of lotic systems occurs with inputs of trace elements despite the influence of dilution with increasing discharge downstream (Chapters 2-5).

Under section 305(b) of the Clean Water Act, states are required to develop and institute monitoring programs that describe water quality conditions and the status of attainment of the designated use of stream segments. The 305(b) report classifies stream segments as supporting, partially supporting, or not supporting a designated use (e.g., drinking water, fishing, swimming). Under section 303(d) of the Clean Water Act, states are required to develop lists of impaired waters that do not meet their designated use. Total maximum daily loads (TMDLs) must be developed for each impaired water body and contaminant loading must be allocated among point and nonpoint sources in the catchment. EPA is currently evaluating tissue-based criteria for protection of wildlife rather than the current water-based standard. Dissolved concentrations, which are used in current water quality standards that trigger a TMDL assessment, are often used to infer tissue concentrations in aquatic organisms. However, dietary pathways of contaminant transfer have been shown to significantly contribute to trace element accumulation in aquatic organisms (Luoma et al. 1992, Mason et al. 2000, Luoma and Rainbow 2005). The results of this dissertation support implementation of tissue-based criteria for trace elements in wildlife and subsequent review of TMDL assessments for rivers.

Literature Cited

Anderson, M. E. 1985. Determination of glutathione and glutathione disulfide in biological samples. Methods in Enzymology **113**:548-555.

Atwell, L., K. A. Hobson, and H. E. Welch. 1998. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. Canadian Journal of Fisheries and Aquatic Sciences **55**:1114-1121.

Barwick, M., and W. Maher. 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**:471-502.

Baudrimont, M., S. Andres, J. Metivaud, Y. Lapaquellerie, F. Ribeyre, N. Maillet, C. Latouche, and A. Boudou. 1999. Field transplantation of the freshwater bivalve Corbicula fluminea along a polymetallic contamination gradient (river Lot, France): II. Metallothionein response to metal exposure. Environmental Toxicology and Chemistry **18**:2472-2477.

Bednar, A. J., J. R. Garbarino, J. F. Ranville, and T. R. Wildeman. 2002. Presence of organoarsenicals used in cotton production in agricultural water and soil of the southern United States. Journal of Agricultural and Food Chemistry **50**:7340-7344.

Beltman, D. J., W. H. Clements, J. Lipton, and D. Cacela. 1999. Benthic invertebrate metals exposure, accumulation, and community-level effects downstream from a hard-rock mine site. Environmental Toxicology and Chemistry **18**:299-307.

Belzile, N., Y. W. Chen, J. M. Gunn, J. Tong, Y. Alarie, T. Delonchamp, and C. Y. Lang. 2006. The effect of selenium on mercury assimilation by freshwater organisms. Canadian Journal of Fisheries and Aquatic Sciences **63**:1-10.

Besser, J. M., W. G. Brumbaugh, T. W. May, S. E. Church, and B. A. Kimball. 2001. Bioavailability of metals in stream food webs and hazards to brook trout (Salvelinus fontinalis) in the upper Animas River watershed, Colorado. Archives of Environmental Contamination and Toxicology **40**:48-59.

Bloom, N. S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Canadian Journal of Fisheries and Aquatic Sciences **49**:1010-1017.

Cabana, G., and J. B. Rasmussen. 1994. Modeling food-chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature **372**:255-257.

Callender, E., and K. C. Rice. 2000. The urban environmental gradient: Anthropogenic influences on the spatial and temporal distributions of lead and zinc in sediments. Environmental Science & Technology **34**:232-238.

Campbell, L. M., R. J. Norstrom, K. A. Hobson, D. C. G. Muir, S. Backus, and A. T. Fisk. 2005. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). Science of the Total Environment **351**:247-263.

Canesi, L., A. Viarengo, C. Leonzio, M. Filippelli, and G. Gallo. 1999. Heavy metals and glutathione metabolism in mussel tissues. Aquatic Toxicology **46**:67-76.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications **8**:559-568.

Cataldo, D., and D. Boltovskoy. 1998. Population dynamics of Corbicula fluminea (Bivalvia) in the Parana River Delta (Argentina). Hydrobiologia **380**:153-163.

Chen, Y. W., N. Belzile, and J. M. Gunn. 2001. Antagonistic effect of selenium on mercury assimilation by fish populations near Sudbury metal smelters? Limnology and Oceanography **46**:1814-1818.

Cherry, D. S., R. K. Guthrie, E. M. Davis, and R. S. Harvey. 1984. Coal ash basin effects (particulates, metals, acidic pH) upon aquatic biota - an 8-year evaluation. Water Resources Bulletin **20**:535-544.

Ciocan, C. M., and J. M. Rotchell. 2004. Cadmium induction of metallothionein isoforms in juvenile and adult mussel (Mytilus edulis). Environmental Science & Technology **38**:1073-1078.

Clements, W. H. 1994. Benthic invertebrate community responses to heavy-metals in the Upper Arkansas River basin, Colorado. Journal of the North American Benthological Society **13**:30-44.

Clements, W. H., D. M. Carlisle, J. M. Lazorchak, and P. C. Johnson. 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecological Applications **10**:626-638.

Clements, W. H., and P. M. Kiffney. 1995. The influence of elevation on benthic community responses to heavy-metals in Rocky-Mountain streams. Canadian Journal of Fisheries and Aquatic Sciences **52**:1966-1977.

Cohen, J. E. 2003. Human population: The next half century. Science **302**:1172-1175.

Costanzo, S. D., M. J. O'Donohue, W. C. Dennison, N. R. Loneragan, and M. Thomas. 2001. A new approach for detecting and mapping sewage impacts. Marine Pollution Bulletin **42**:149-156.

Couillard, Y., P. G. C. Campbell, A. Tessier, J. Pellerinmassicotte, and J. C. Auclair. 1995. Field transplantation of a fresh-water bivalve, Pyganodon grandis, across a metal contamination gradient .1. Temporal changes in metallothionein and metal (Cd, Cu, and Zn) concentrations in soft-tissues. Canadian Journal of Fisheries and Aquatic Sciences **52**:690-702.

Crutchfield, J. U. 2000. Recovery of a power plant cooling reservoir ecosystem from selenium bioaccumulation. Environmental Science & Policy **3**:145-163.

Daughton, C. G., and T. A. Ternes. 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environmental Health Perspectives **107**:907-938.

Deniro, M. J., and S. Epstein. 1978. Influence of diet on distribution of carbon isotopes in animals. Geochimica Et Cosmochimica Acta **42**:495-506.

Dodson, S. I., R. A. Lillie, and S. Will-Wolf. 2005. Land use, water chemistry, aquatic vegetation, and zooplankton community structure of shallow lakes. Ecological Applications **15**:1191-1198.

Driscoll, C. T., V. Blette, C. Yan, C. L. Schofield, R. Munson, and J. Holsapple. 1995. The role of dissolved organic-carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes. Water Air and Soil Pollution **80**:499-508.

Farag, A. M., D. F. Woodward, J. N. Goldstein, W. Brumbaugh, and J. S. Meyer. 1998. Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River Basin, Idaho. Archives of Environmental Contamination and Toxicology **34**:119-127.

Fitzgerald, W. F., D. R. Engstrom, R. P. Mason, and E. A. Nater. 1998. The case for atmospheric mercury contamination in remote areas. Environmental Science & Technology **32**:1-7.

Frick, E. A., D. J. Hippe, G. R. Buell, C. A. Couch, E. H. Hopkins, D. J. Wangsness, and J. W. Garrett. 1998. Water quality in the Apalachicola-Chattahoochee-Flint River Basin, Georgia, Alabama, and Florida, 1992-1995. USGS Circular Report 1164.

Froelich, P. N., and P. Lesley. 2001. Tracing germanium contamination from coal-fired power plants along the Chattahoochee River-Apalachicola River: implications for the toxic metalloids arsenic and selenium. *in* K. J. Hatcher, editor. Proceedings of the Georgia Water Resources Conference. Institute of Ecology, University of Georgia, Athens, GA.

Graney, R. L., D. S. Cherry, and J. Cairns. 1983. Heavy-metal indicator potential of the asiatic clam (Corbicula-fluminea) in artificial stream systems. Hydrobiologia **102**:81-88.

Grigal, D. F., R. K. Kolka, J. A. Fleck, and E. A. Nater. 2000. Mercury budget of an upland-peatland watershed. Biogeochemistry **50**:95-109.

Guthrie, R. K., and D. S. Cherry. 1979. Trophic level accumulation of heavy-metals in a coal ash basin drainage system. Water Resources Bulletin **15**:244-248.

Hamilton, S. J. 2002. Rationale for a tissue-based selenium criterion for aquatic life. Aquatic Toxicology **57**:85-100.

Hamilton, S. J. 2004. Review of selenium toxicity in the aquatic food chain. Science of the Total Environment **326**:1-31.

Hamilton, S. J., and K. J. Buhl. 2004. Selenium in water, sediment, plants, invertebrates, and fish in the blackfoot river drainage. Water Air and Soil Pollution **159**:2-34.

Harding, J. S., E. F. Benfield, P. V. Bolstad, G. S. Helfman, and E. B. D. Jones. 1998. Stream biodiversity: The ghost of land use past. Proceedings of the National Academy of Sciences of the United States of America **95**:14843-14847.

Heikoop, J. M., M. J. Risk, A. V. Lazier, E. N. Edinger, J. Jompa, G. V. Limmon, J. J. Dunn, D. R. Browne, and H. P. Schwarcz. 2000. Nitrogen-15 signals of anthropogenic nutrient loading in reef corals. Marine Pollution Bulletin **40**:628-636.

Hopkins, W. A., M. T. Mendonca, C. L. Rowe, and J. D. Congdon. 1998. Elevated trace element concentrations in southern toads, Bufo terrestris, exposed to coal combustion waste. Archives of Environmental Contamination and Toxicology **35**:325-329.

Hopkins, W. A., J. W. Snodgrass, J. H. Roe, B. P. Jackson, J. C. Gariboldi, and J. D. Congdon. 2000. Detrimental effects associated with trace element uptake in lake chubsuckers (Erimyzon sucetta) exposed to polluted sediments. Archives of Environmental Contamination and Toxicology **39**:193-199.

Hopkins, W. A., B. P. Staub, J. A. Baionno, B. P. Jackson, J. H. Roe, and N. B. Ford. 2004a. Trophic and maternal transfer of selenium in brown house snakes (Lamprophis fuliginosus). Ecotoxicology and Environmental Safety **58**:285-293.

Hopkins, W. A., B. P. Staub, J. W. Snodgrass, B. E. Taylor, A. E. DeBiase, J. H. Roe, B. P. Jackson, and J. D. Congdon. 2004b. Responses of benthic fish exposed to contaminants in outdoor microcosms - examining the ecological relevance of previous laboratory toxicity tests. Aquatic Toxicology **68**:1-12.

Horowitz, A. J., M. Meybeck, Z. Idlafkih, and E. Biger. 1999. Variations in trace element geochemistry in the Seine River Basin based on floodplain deposits and bed sediments. Hydrological Processes **13**:1329-1340.

Hull, M. S., D. S. Cherry, and T. C. Merricks. 2004. Effect of cage design on growth of transplanted Asian clams: Implications for assessing bivalve responses in streams. Environmental Monitoring and Assessment **96**:1-14.

Hull, M. S., D. S. Cherry, and R. J. Neves. 2006. Use of bivalve metrics to quantify influences of coal-related activities in the Clinch River watershed, virginia. Hydrobiologia **556**:341-355.

Irato, P., G. Santovito, A. Cassini, E. Piccinni, and V. Albergoni. 2003. Metal accumulation and binding protein induction in Mytilus galloprovincialis, Scapharca inaequivalvis, and Tapes philippinarum from the lagoon of Venice. Archives of Environmental Contamination and Toxicology **44**:476-484.

Jackson, B. P., P. L. S. Allen, W. A. Hopkins, and P. M. Bertsch. 2002. Trace element speciation in largemouth bass (Micropterus salmoides) from a fly ash settling basin by liquid chromatography-ICP-MS. Analytical and Bioanalytical Chemistry **374**:203-211.

Jackson, B. P., and P. M. Bertsch. 2001. Determination of arsenic speciation in poultry wastes by IC-ICP-MS. Environmental Science & Technology **35**:4868-4873.

Jackson, B. P., P. M. Bertsch, M. L. Cabrera, J. J. Camberato, J. C. Seaman, and C. W. Wood. 2003. Trace element speciation in poultry litter. Journal of Environmental Quality **32**:535-540.

Karvelas, M., A. Katsoyiannis, and C. Samara. 2003. Occurrence and fate of heavy metals in the wastewater treatment process. Chemosphere **53**:1201-1210.

Kesler, D. H. 2004. Influence of a lentic area on condition indices of Corbicula fluminea in the Wolf River, Tennessee. Journal of Freshwater Ecology **19**:445-453.

Kolka, R. K., D. F. Grigal, E. S. Verry, and E. A. Nater. 1999a. Mercury and organic carbon relationships in streams draining forested upland peatland watersheds. Journal of Environmental Quality **28**:766-775.

Kolka, R. K., E. A. Nater, D. F. Grigal, and E. S. Verry. 1999b. Atmospheric inputs of mercury and organic carbon into a forested upland bog watershed. Water Air and Soil Pollution **113**:273-294.

Kolpin, D. W., E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber, and H. T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. Environmental Science & Technology **36**:1202-1211.

Lemly, A. D. 1985. Toxicology of selenium in a fresh-water reservoir - Implications for environmental-hazard evaluation and safety. Ecotoxicology and Environmental Safety **10**:314-338.

Lemly, A. D. 1993a. Guidelines for evaluating selenium data from aquatic monitoring and assessment studies. Environmental Monitoring and Assessment **28**:83-100.

Lemly, A. D. 1993b. Teratogenic effects of selenium in natural-populations of fresh-water fish. Ecotoxicology and Environmental Safety **26**:181-204.

Lemly, A. D. 1997. Ecosystem recovery following selenium contamination in a freshwater reservoir. Ecotoxicology and Environmental Safety **36**:275-281.

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

Lenat, D. R., and J. K. Crawford. 1994. Effects of land-use on water-quality and aquatic biota of 3 North-Carolina Piedmont streams. Hydrobiologia **294**:185-199.

Lesley, P., and P. N. Froelich. 2003. Arsenic, selenium, and antimony: from coal fired power plants to the Chattahoochee River. *in* K. J. Hatcher, editor. Proceedings of the 2003 Georgia Water Resources Conference. University of Georgia, Athens, GA.

Loeffler, G., J. L. Meyer, H. Trammell, and S. Holmbeck-Pelham. 2003. Fish consumption patterns along the Upper Chattahoochee River. *in* K. J. Hatcher, editor. Proceedings of the 2003 Georgia Water Resources Conference. University of Georgia, Athens, GA.

Lohner, T. W., R. J. Reash, V. E. Willet, and L. A. Rose. 2001a. Assessment of tolerant sunfish populations (Lepomis sp.) inhabiting selenium-laden coal ash effluents - 1. Hematological and population level assessment. Ecotoxicology and Environmental Safety **50**:203-216.

Lohner, T. W., R. J. Reash, and M. Williams. 2001b. Assessment of tolerant sunfish populations (Lepomis sp.) inhabiting selenium-laden coal ash effluents - 2. Tissue biochemistry evaluation. Ecotoxicology and Environmental Safety **50**:217-224.

Luoma, S. N., C. Johns, N. S. Fisher, N. A. Steinberg, R. S. Oremland, and J. R. Reinfelder. 1992. Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. Environmental Science & Technology **26**:485-491.

Luoma, S. N., and P. S. Rainbow. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environmental Science & Technology **39**:1921-1931.

Mason, R. P., W. F. Fitzgerald, and F. M. M. Morel. 1994. The biogeochemical cycling of elemental mercury - Anthropogenic influences. Geochimica Et Cosmochimica Acta **58**:3191-3198.

Mason, R. P., J. M. Laporte, and S. Andres. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. Archives of Environmental Contamination and Toxicology **38**:283-297.

McArthur, J. V., and R. C. Tuckfield. 2000. Spatial patterns in antibiotic resistance among stream bacteria: Effects of industrial pollution. Applied and Environmental Microbiology **66**:3722-3726.

McMahon, R. F. 1991. Mollusca: Bivalvia. *in* J. H. Thorp and A. P. Covich, editors. Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego.

Meyer, J. L., M. J. Paul, and W. K. Taulbee. 2005. Stream ecosystem function in urbanizing landscapes. Journal of the North American Benthological Society **24**:602-612.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of N-15 along food-chains - Further evidence and the relation between delta-N-15 and animal age. Geochimica Et Cosmochimica Acta **48**:1135-1140.

Morel, F. M. M., A. M. L. Kraepiel, and M. Amyot. 1998. The chemical cycle and bioaccumulation of mercury. Annual Review of Ecology and Systematics **29**:543-566.

Nagle, R. D., C. L. Rowe, and J. D. Congdon. 2001. Accumulation and selective maternal transfer of contaminants in the turtle Trachemys scripta associated with coal ash deposition. Archives of Environmental Contamination and Toxicology **40**:531-536.

NRC. 2006. Managing coal combustion residues in mines. The National Academies Press, Washington, D.C.

Orr, P. L., K. R. Guiguer, and C. K. Russel. 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. Ecotoxicology and Environmental Safety **63**:175-188.

Paul, M. J., and J. L. Meyer. 2001. Streams in the urban landscape. Annual Review of Ecology and Systematics **32**:333-365.

Pedlar, R. M., M. D. Ptashynski, K. G. Wautier, R. E. Evans, C. L. Baron, and J. F. Klaverkamp. 2002. The accumulation, distribution, and toxicological effects of dietary arsenic exposure in lake whitefish (Coregonus clupeaformis) and lake trout (Salvelinus namaycush). Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **131**:73-91.

Ravichandran, M. 2004. Interactions between mercury and dissolved organic matter - a review. Chemosphere **55**:319-331.

Reinfelder, J. R., W. X. Wang, S. N. Luoma, and N. S. Fisher. 1997. Assimilation efficiencies and turnover rates of trace elements in marine bivalves: a comparison of oysters, clams and mussels. Marine Biology **129**:443-452.

Ringwood, A. H., D. E. Conners, C. J. Keppler, and A. A. DiNovo. 1999. Biomarker studies with juvenile oysters (Crassostrea virginica) deployed in-situ. Biomarkers **4**:400-414.

Rose, S. 2002. Comparative major ion geochemistry of Piedmont streams in the Atlanta, Georgia region: possible effects of urbanization. Environmental Geology **42**:102-113.

Rose, S., M. S. Crean, D. K. Sheheen, and A. M. Ghazi. 2001. Comparative zinc dynamics in Atlanta metropolitan region stream and street runoff. Environmental Geology **40**:983-992.

Rosi-Marshall, E. J. 2002. Quality of suspended fine particulate matter and its role as a conduit for metals in riverine food webs. Ph.D. The University of Georgia, Athens.

Rowe, C. L., W. A. Hopkins, and J. D. Congdon. 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: A review. Environmental Monitoring and Assessment **80**:207-276.

Rowe, C. L., W. A. Hopkins, C. Zehnder, and J. D. Congdon. 2001. Metabolic costs incurred by crayfish (Procambarus acutus) in a trace element-polluted habitat: further evidence of similar responses among diverse taxonomic groups. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **129**:275-283.

Roy, A. H., A. D. Rosemond, M. J. Paul, D. S. Leigh, and J. B. Wallace. 2003. Stream macroinvertebrate response to catchment urbanisation (Georgia, USA). Freshwater Biology **48**:329-346.

Rozan, T. F., and G. Benoit. 2001. Mass balance of heavy metals in New Haven Harbor, Connecticut: Predominance of nonpoint sources. Limnology and Oceanography **46**:2032-2049.

Rudd, J. W. M., M. A. Turner, A. Furutani, B. E. Townsend, and A. Swick. 1980. Dynamics of selenium in mercury-contaminated experimental fresh-water ecosystems. Canadian Journal of Fisheries and Aquatic Sciences **37**:848-857.

Ruhl, P. M., and K. E. Smith. 1996. Water-quality assessment of the Albemarle-Pamlico drainage basin, North Carolina and Virginia - Trace elements in Asiatic clam (*Corbicula fluminea*) soft tissues and redbreast sunfish (*Lepomis auritus*) livers, 1992-93. Open-File Report 96-334.

Sandhu, S. S., G. L. Mills, and K. S. Sajwan. 1993. Leachability of Ni, Cd, Cr, and As from coal ash impoundments of different ages on the Savannah River Site. *in* R. K. Keefer and K. S. Sajwan, editors. Trace elements in coal and coal combustion residues. CRC Press, Boca Raton, FL.

Simmons, D. B. D., and D. Wallschlager. 2005. A critical review of the biogeochemistry and ecotoxicology of selenium in lotic and lentic environments. Environmental Toxicology and Chemistry **24**:1331-1343.

Sonesten, L. 2001. Mercury content in roach (Rutilus rutilus L.) in circumneutral lakes - effects of catchment area and water chemistry. Environmental Pollution **112**:471-481.

Sonesten, L. 2003. Catchment area composition and water chemistry heavily affects mercury levels in perch (Perca fluviatilis L.) in circumneutral lakes. Water Air and Soil Pollution **144**:117-139.

Soucek, D. J., T. S. Schmidt, and D. S. Cherry. 2001. In situ studies with Asian clams (Corbicula fluminea) detect acid mine drainage and nutrient inputs in low-order streams. Canadian Journal of Fisheries and Aquatic Sciences **58**:602-608.

Stewart, A. R., S. N. Luoma, C. E. Schlekat, M. A. Doblin, and K. A. Hieb. 2004. Food web pathway determines how selenium affects aquatic ecosystems: A San Francisco Bay case study. Environmental Science & Technology **38**:4519-4526.

Stohs, S. J., and D. Bagchi. 1995. Oxidative mechanisms in the toxicity of metal-ions. Free Radical Biology and Medicine **18**:321-336.

Turner, M. A., and J. W. M. Rudd. 1983. The English Wabigoon River system .3. Selenium in lake enclosures - Its geochemistry, bioaccumulation, and ability to reduce mercury bioaccumulation. Canadian Journal of Fisheries and Aquatic Sciences **40**:2228-2240.

Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumer delta C-13 and delta N-15 and the trophic position of aquatic consumers. Ecology **80**:1395-1404.

Vander Zanden, M. J., B. J. Shuter, N. Lester, and J. B. Rasmussen. 1999. Patterns of food chain length in lakes: A stable isotope study. American Naturalist **154**:406-416.

Vaughn, C. C., and C. C. Hakenkamp. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology **46**:1431-1446.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P. Morgan. 2005. The urban stream syndrome: current knowledge and the search for a cure. Journal of the North American Benthological Society **24**:706-723.

Yan, T., L. H. Teo, and Y. M. Sin. 1997. Effects of mercury and lead on tissue glutathione of the green mussel, Perna viridis L. Bulletin of Environmental Contamination and Toxicology **58**:845-850.

APPENDIX A

SUPPLEMENTAL MATERIAL TO CHAPTER 2

Appendix A.1. Concentrations of cations and anions at all study sites. BDL = below detection limit.

Site Name	Land use	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)	Sodium (µg/L)	Chloride (mg/L)	Nitrate (mg/L)	Sulfate (mg/L)
Broad River	Forest	2.965	0.321	3.564	24.943	3.380	2.992	3.043
Flat Shoals Creek	Forest	2.735	0.655	2.068	129.383	2.760	0.825	0.857
Snake Creek	Forest	1.776	0.065	1.199	19.582	3.894	1.057	0.816
East Fork Little River	Agriculture	3.567	0.504	1.748	26.346	4.125	5.596	1.442
Wahoo Creek	Agriculture	4.673	0.479	2.427	30.681	3.138	2.692	1.826
West Fork Little River	Agriculture	4.366	0.648	2.064	15.762	4.954	12.263	1.559
Nancy Creek	Urban	8.887	0.237	4.376	41.393	7.763	3.407	8.591
Peachtree	Urban	15.109	1.208	3.285	18.680	11.980	3.260	11.048
Sope Creek	Urban	8.549	0.728	1.952	63.119	11.841	3.345	4.996
Plant McDonough	Coal	8.811	0.280	4.195	33.777	17.082	9.557	10.691
Plant Wansley	Coal	86.609	69.160	10.623	73.492	10.493	BDL	203.024
Plant Yates	Coal	10.895	0.411	4.287	27.213	19.080	9.541	26.729
Anneewakee Creek	Wastewater	4.685	0.448	3.212	4.454	10.178	5.501	7.291
Big Creek	Wastewater	5.951	1.124	0.855	35.047	16.771	3.131	3.970
Suwanee Creek	Wastewater	10.050	0.308	4.253	114.562	11.928	7.098	9.418

Appendix A.2. Trace element concentrations (μg g^{-1} dry mass) in *Corbicula fluminea* tissue grouped by site type and study location.

ClamID	Site Name	Site Type	V 51	Mn 55	Fe 57	Co 59	Ni 60	Cu 65	Zn 68	As 75	Se 77	Sr 88	Cd 114	Hg 202	Pb 208
Broad1	Broad River	Forest	0.3398	36.3560	373.9084	1.2709	0.5668	41.6786	150.0786	3.3527	3.7596	10.0442	0.7766	0.1566	0.1454
Broad11	Broad River	Forest	0.6820	154.0201	686.9756	1.3605	0.9655	26.7964	172.6186	2.7778	4.3496	16.1654	0.8080	0.1493	0.4139
Broad2	Broad River	Forest	1.4608	43.5753	1673.1960	1.7640	0.6836	39.4944	237.3483	3.4217	4.2073	14.6313	0.7535	0.1062	0.4883
Broad3	Broad River	Forest	1.4047	39.0494	749.4298	1.5207	1.0129	31.6452	197.3879	3.1586	3.5947	12.4078	0.9051	0.1169	0.5140
Broad4	Broad River	Forest	0.9652	137.0002	539.9043	1.5359	0.7445	31.9509	172.5525	2.9298	3.4524	10.8136	0.8024	0.1386	0.3090
Broad6	Broad River	Forest	0.3841	25.8827	540.4199	1.5397	0.9386	34.9341	211.9342	3.4649	4.6725	20.4265	0.8854	0.1594	0.2136
Broad7	Broad River	Forest	0.3259	21.8093	394.4628	1.4393	0.6822	27.4242	199.2433	3.0552	3.8038	12.0826	1.0002	0.1333	0.2046
Broad8	Broad River	Forest	0.2958	26.7315	371.2275	1.1744	0.6315	28.5060	181.9456	3.1914	4.0204	12.4294	0.9220	0.1298	0.1904
Broad9	Broad River	Forest	0.4419	26.0890	483.3527	1.3875	0.5684	25.3097	181.3696	2.5859	3.4936	11.4727	0.7641	0.1100	0.2941
FSC1	Flat Shoals Creek	Forest	0.2979	501.5531	813.5876	4.4735	2.1113	43.3296	228.7069	3.0518	4.7556	67.5503	1.4828	0.6921	0.1654
FSC2	Flat Shoals Creek	Forest	0.4453	656.5517	983.4027	5.3999	2.4372	34.4951	264.3499	2.5473	4.5376	76.7402	2.6427	0.5523	0.2930
FSC3	Flat Shoals Creek	Forest	0.4208	646.1623	1068.0330	6.2514	2.7993	41.0184	301.0058	3.3720	5.3582	120.6655	2.4647	0.7317	0.2380
FSC4	Flat Shoals Creek	Forest	0.7192	602.9544	1012.6817	3.8031	1.8098	30.9538	190.3552	2.3967	3.8633	56.5333	2.1000	0.5008	0.7359
FSC5	Flat Shoals Creek	Forest	0.7508	728.5978	1180.0402	5.0438	2.3185	31.1479	272.5940	2.4325	4.3773	86.4891	1.9255	0.3302	0.4145
FSC6	Flat Shoals Creek	Forest	0.6303	520.6471	1076.1337	3.3513	2.2911	34.9664	242.3110	2.5364	4.5134	67.2403	2.0883	0.5629	0.5706
FSC7	Flat Shoals Creek	Forest	4.7241	957.8754	4211.6633	5.2908	3.1886	33.0943	232.2074	3.1185	4.6062	74.4915	2.0522	0.5023	1.8754
FSC8	Flat Shoals Creek	Forest	0.5962	604.0965	1002.9672	5.3441	2.1673	33.9560	240.1851	2.6431	4.4865	72.1922	2.4393	0.5349	0.3292
FSC9	Flat Shoals Creek	Forest	0.7767	831.6868	1342.1937	3.8883	2.3119	37.8391	240.8979	3.0824	5.2131	84.0125	1.9492	0.6738	0.5031
Snake1	Snake Creek	Forest	0.2096	25.1649	405.8594	2.6603	0.8078	41.5556	358.9499	3.9179	4.4748	10.0264	2.4525	0.3020	0.3357
Snake11	Snake Creek	Forest	0.2665	33.2080	694.3123	3.1688	1.3133	33.1342	373.2302	2.5830	3.1048	12.3779	3.1924	0.2893	0.9728
Snake12	Snake Creek	Forest	0.3008	31.4097	625.8636	1.8893	1.2600	36.5196	358.5538	3.1205	3.9500	9.0720	3.0025	0.3131	0.5587
Snake2	Snake Creek	Forest	0.3138	30.2201	539.1715	2.4100	1.0692	35.9351	344.8216	2.7044	3.3525	9.9480	2.4275	0.3329	0.5729
Snake3	Snake Creek	Forest	0.2067	25.6275	501.6777	1.8912	0.9794	52.8766	294.7716	3.7019	4.1403	10.2592	2.6674	0.7473	0.6025
Snake5	Snake Creek	Forest	0.2806	37.6648	630.0919	4.2106	1.1837	42.6803	468.3877	3.7408	3.5695	16.9302	2.4809	0.2572	0.6643
Snake6	Snake Creek	Forest	0.2884	27.6899	401.5914	1.6214	0.6979	35.4299	216.5457	3.1690	3.5175	5.7102	2.4204	0.4975	0.7022
Snake7	Snake Creek	Forest	0.4081	32.3095	666.0763	2.2797	0.9379	30.4539	314.7016	2.8604	4.5980	9.1177	3.0107	0.2911	0.6206
Snake8	Snake Creek	Forest	0.4753	44.3487	606.9104	1.5761	0.9278	30.7882	279.5109	2.7739	2.8400	7.7484	2.1494	0.2001	0.7093
EFR1	East Fork Little River	Agriculture	0.1234	23.7306	387.9384	1.0824	0.6222	36.8166	217.9983	2.0913	3.2793	21.4130	1.8641	0.3237	0.6920
EFR10	East Fork Little River	Agriculture	0.1734	30.3825	376.4830	1.3298	0.4598	33.5031	240.8092	1.9628	3.2221	19.5946	1.9350	0.2979	0.5248
EFR2	East Fork Little River	Agriculture	0.1291	21.0457	327.5456	1.2766	0.6107	37.3236	231.4051	1.9188	2.9819	17.3187	1.7097	0.2026	0.3658
EFR3	East Fork Little River	Agriculture	1.2571	31.3625	965.9491	1.9975	0.8524	38.8210	299.5414	2.4303	3.9063	26.2572	2.7744	0.3166	0.8658
EFR4	East Fork Little River	Agriculture	0.1671	27.2506	325.6845	1.3363	0.6755	36.6080	244.7973	1.9650	3.1786	21.2460	1.9113	0.2088	0.4100

Appendix A.2. Continued.

ClamID	Site Name	Site Type	V 51	Mn 55	Fe 57	Co 59	Ni 60	Cu 65	Zn 68	As 75	Se 77	Sr 88	Cd 114	Hg 202	Pb 208
EFR5	East Fork Little River	Agriculture	0.1543	27.1728	493.1911	0.9506	0.4226	42.0660	231.1429	2.3565	3.8645	18.0614	3.2260	0.2626	1.1268
EFR6	East Fork Little River	Agriculture	0.1272	32.0069	486.6781	1.4721	0.7698	43.3465	243.3445	1.9883	3.3524	22.6780	2.0402	0.2232	0.8008
EFR8	East Fork Little River	Agriculture	0.1806	19.8151	457.0426	0.9183	0.3998	37.0770	210.7883	2.3074	3.5805	16.8006	2.7834	0.2380	0.8090
EFR9	East Fork Little River	Agriculture	0.1861	27.5472	473.2927	1.3821	0.5975	38.3211	276.8883	2.0341	3.1119	26.1193	1.9843	0.2241	0.5929
WC1	Wahoo Creek	Agriculture	0.2526	25.8522	572.1588	2.0361	0.9318	36.8441	243.6030	2.2708	4.3431	18.4164	1.0431	0.2667	0.2912
WC10	Wahoo Creek	Agriculture	0.1828	29.2747	445.6565	1.6199	0.9366	37.2935	213.1477	2.3454	4.1585	19.1058	0.8057	0.3083	0.2465
WC2	Wahoo Creek	Agriculture	0.2643	24.4313	481.6486	2.2789	0.7922	42.0517	227.2950	2.9658	4.5344	18.2198	1.1026	0.3347	0.2487
WC4	Wahoo Creek	Agriculture	0.2114	30.3856	482.7431	1.9608	0.8825	38.6990	233.5699	2.3150	4.3067	18.5596	1.0520	0.2361	0.4260
WC5	Wahoo Creek	Agriculture	17.1555	42.9063	8071.8701	2.9129	1.1473	28.8691	257.7265	2.3362	3.6992	22.9935	0.9701	0.2794	0.4624
WC6	Wahoo Creek	Agriculture	0.5797	33.2816	771.3413	2.7698	1.2338	39.2808	286.8329	2.3355	4.2273	23.7925	1.3626	0.3381	0.9302
WC7	Wahoo Creek	Agriculture	0.2869	25.9544	614.5859	2.8275	1.1606	46.1533	269.6163	3.0708	5.0913	21.2922	1.2553	0.4180	0.3582
WC8	Wahoo Creek	Agriculture	0.2992	27.0341	505.5735	2.2084	0.7724	30.4340	252.0939	2.3949	4.4931	19.9322	0.9756	0.2476	0.2708
WC9	Wahoo Creek	Agriculture	0.6464	51.2844	811.6717	2.7169	1.5304	35.1887	238.0888	2.0259	4.0434	20.0021	1.1818	0.3489	0.4605
WFR1	West Fork Little River	Agriculture	0.4328	17.8848	523.2117	1.2091	0.8335	37.3815	326.1254	2.3448	3.8797	20.9996	0.9857	0.2883	0.6252
WFR11	West Fork Little River	Agriculture	0.4352	29.3212	658.8868	1.1566	0.6785	35.0926	273.8010	1.9344	3.6375	21.8137	1.3513	0.3554	0.4807
WFR2	West Fork Little River	Agriculture	12.3958	37.2751	3403.8654	1.6918	0.9113	39.2196	274.1892	2.4522	4.1414	23.6882	1.1740	0.3142	0.5528
WFR4	West Fork Little River	Agriculture	0.4513	37.6014	610.7503	1.0057	0.6380	32.8871	289.2295	1.9721	3.2734	18.9827	1.4259	0.2372	0.3903
WFR5	West Fork Little River	Agriculture	0.5164	33.0274	597.5820	2.6066	1.0306	39.0461	330.6457	2.3764	3.4478	21.4393	0.9148	0.3755	0.4900
WFR6	West Fork Little River	Agriculture	0.9534	40.3863	837.4179	1.2799	0.8889	34.0235	334.3176	2.1550	4.5117	23.0762	2.5065	0.3322	0.6219
WFR7	West Fork Little River	Agriculture	0.5758	26.3266	673.4801	1.1302	0.8640	34.7201	247.8422	2.0459	3.4840	29.9433	2.4459	0.2382	0.3853
WFR8	West Fork Little River	Agriculture	0.2777	25.0848	453.6434	1.2676	0.6971	41.8069	309.8999	2.2703	3.4170	15.7349	0.7568	0.2448	0.4569
WFR9	West Fork Little River	Agriculture	0.3832	27.1646	565.0300	2.1377	1.0822	34.2674	359.8351	2.0290	3.6610	27.1771	1.8079	0.2466	0.4955
Nancy1	Nancy Creek	Urban	0.1410	16.6858	266.2665	1.3241	0.7479	44.7201	345.3621	3.4275	4.0967	13.6292	1.4675	0.1165	0.5476
Nancy10	Nancy Creek	Urban	0.2970	28.1267	320.4826	1.0341	0.8760	33.5261	271.7866	2.7203	2.7811	12.2320	1.7281	0.1363	0.5217
Nancy12	Nancy Creek	Urban	0.2026	26.8665	293.9758	1.1170	0.9812	34.9424	278.8274	2.5119	3.2629	12.5539	1.5331	0.1572	2.1536
Nancy2	Nancy Creek	Urban	0.2774	18.3627	363.0293	1.1634	0.9215	36.5885	386.8670	3.0718	3.1879	13.8240	1.2798	0.1406	0.5153
Nancy3	Nancy Creek	Urban	0.2893	22.7442	353.8664	0.8805	0.8432	36.4848	271.0085	2.8159	2.8840	12.0593	1.2326	0.1353	0.5985
Nancy5	Nancy Creek	Urban	0.2938	23.0785	307.3629	1.8689	0.9178	37.6350	401.6355	3.1402	3.2006	12.7541	1.8683	0.1247	0.5287
Nancy6	Nancy Creek	Urban	0.3786	24.6401	410.5435	1.1242	0.9675	39.5811	336.5367	4.0079	3.8075	14.6046	1.8780	0.1522	0.6191
Nancy7	Nancy Creek	Urban	0.1896	19.2961	263.1058	0.8170	0.8153	36.2490	246.5619	2.8552	3.1612	9.5995	1.5379	0.1385	1.0124
Nancy9	Nancy Creek	Urban	0.1530	18.7438	245.5474	0.9001	0.7889	30.6182	190.9680	2.6257	2.9300	14.2486	1.4086	0.1044	0.9835
Peach10	Peachtree Creek	Urban	0.4482	32.8059	716.3842	1.5272	1.4166	67.9335	493.0041	2.7154	4.7546	12.6387	3.4644	0.2406	0.9680

Appendix A.2. Continued.

ClamID	Site Name	Site Type	V 51	Mn 55	Fe 57	Co 59	Ni 60	Cu 65	Zn 68	As 75	Se 77	Sr 88	Cd 114	Hg 202	Pb 208
Peach11	Peachtree Creek	Urban	0.5134	35.0690	726.6940	1.7144	1.1729	58.0391	563.3532	2.7570	5.1299	13.9805	4.7593	0.2102	1.3374
Peach12	Peachtree Creek	Urban	0.4205	30.4516	592.2948	1.4908	0.9132	62.2861	490.6003	2.7126	4.5110	11.2206	4.4828	0.1985	0.9993
Peach2	Peachtree Creek	Urban	0.4582	33.9436	588.3589	1.1025	0.9090	61.0914	380.7577	3.2488	4.5906	8.7095	3.8871	0.2448	0.9002
Peach3	Peachtree Creek	Urban	0.4435	29.8581	703.3389	1.2677	0.9939	53.5229	472.7018	2.6046	5.1075	9.6493	3.4465	0.1888	1.0271
Peach6	Peachtree Creek	Urban	0.3799	30.2902	500.7379	1.5990	1.6213	65.3526	648.1177	2.4682	4.7077	13.1966	4.0501	0.1823	1.2465
Peach7	Peachtree Creek	Urban	0.4584	23.7253	585.3059	1.1344	0.9598	64.7249	363.1788	2.5901	4.2473	7.7319	3.7926	0.1974	0.8409
Peach8	Peachtree Creek	Urban	0.3998	32.8529	591.4972	1.7132	0.9833	76.5311	374.9105	2.8033	4.5981	11.2864	5.0835	0.2630	1.1936
Peach9	Peachtree Creek	Urban	0.4623	30.2945	669.1836	1.2832	1.1949	63.1045	420.7503	2.4976	4.2341	9.1354	3.7752	0.2004	1.7697
Sope1	Sope Creek	Urban	0.5106	27.2307	713.9162	1.0803	0.8578	41.7420	162.3000	3.0680	4.2113	6.4689	2.5259	0.2245	0.9731
Sope2	Sope Creek	Urban	0.6724	36.7317	1314.4954	1.8596	1.7957	50.1316	256.3079	4.1139	5.3779	14.9299	3.1850	0.2719	1.6142
Sope3	Sope Creek	Urban	0.4924	39.0882	876.6135	1.4630	0.9122	43.6082	228.5801	3.1887	4.7196	9.5781	2.2253	0.2311	0.9307
Sope4	Sope Creek	Urban	0.6604	39.5781	972.5190	1.8116	1.2314	46.2234	284.1212	3.3589	4.8006	8.7671	2.6514	0.1704	1.1029
Sope5	Sope Creek	Urban	0.5436	44.2187	964.2414	2.2857	1.3822	46.4686	402.5174	3.4818	5.1543	11.5727	2.5611	0.1989	1.1058
Sope6	Sope Creek	Urban	0.5752	34.6684	1086.0367	1.3609	0.9275	37.2000	231.9824	3.0984	4.8493	8.0260	2.6527	0.2352	1.0529
Sope7	Sope Creek	Urban	0.4745	36.4824	992.4609	1.7902	1.3011	37.7721	412.1219	2.9773	4.7049	13.7606	2.5029	0.1724	1.1340
Sope8	Sope Creek	Urban	0.4799	32.9312	1077.0853	1.5564	1.5626	36.5693	304.9246	3.2244	4.4796	14.8671	3.0867	0.2312	1.1730
Sope9	Sope Creek	Urban	1.1377	45.0987	1971.5688	1.5112	1.1453	41.5330	381.7522	3.2864	4.7467	10.8289	1.8977	0.1769	1.5231
MCD1	Plant McDonough	Coal	0.6593	22.6361	569.7497	1.7674	1.1332	55.8387	422.0887	2.3854	5.0130	12.7402	2.0886	0.1211	0.5824
MCD12	Plant McDonough	Coal	0.1825	15.3908	450.1250	1.5303	1.3164	44.8251	444.0381	1.6034	4.6245	17.5643	3.6998	0.0956	0.3452
MCD2	Plant McDonough	Coal	0.3821	20.8375	654.8655	3.3905	1.4822	67.8814	523.4969	2.4906	4.6434	16.7350	1.7565	0.1318	0.5200
MCD3	Plant McDonough	Coal	0.1655	22.0644	372.6078	3.5135	1.4081	54.9578	743.4928	2.1650	4.9271	18.3250	1.8828	0.1017	0.3554
MCD4	Plant McDonough	Coal	0.1825	18.1071	345.2164	2.4578	1.3650	64.3499	533.2117	2.3712	4.7198	13.7525	2.0933	0.0981	0.2942
MCD5	Plant McDonough	Coal	0.2307	31.2211	397.9595	2.8743	1.2584	80.1722	546.7612	2.6965	5.2352	17.3919	1.8221	0.1017	0.3614
MCD7	Plant McDonough	Coal	0.2414	18.0439	436.8467	1.9640	1.1860	61.1176	425.1140	2.1736	3.9774	16.2825	1.6312	0.1413	0.4411
MCD8	Plant McDonough	Coal	0.1794	7.7801	355.9462	2.1342	1.0830	55.8019	391.8979	2.2941	3.5056	11.5950	1.2192	0.1700	0.5653
MCD9	Plant McDonough	Coal	0.2281	22.6443	406.3121	3.5066	1.5039	66.2457	555.5368	2.1683	4.0854	17.5833	2.2068	0.1411	0.6682
WAN1	Plant Wansley	Coal	0.5446	61.2661	373.0720	1.4981	1.7377	39.3769	559.7810	4.8730	9.6793	20.3620	2.3427	0.1300	0.4518
WAN10	Plant Wansley	Coal	0.8314	47.7875	487.1086	1.3743	1.5407	39.3627	576.0919	5.0440	10.3701	21.7885	2.4056	0.1131	0.6429
WAN2	Plant Wansley	Coal	1.0052	59.6756	729.0570	1.3303	1.4917	51.3954	546.9896	5.2234	10.3768	20.9266	2.4063	0.1438	0.8035
WAN3	Plant Wansley	Coal	0.7500	38.5310	468.3847	1.4871	2.5243	38.5915	588.7736	4.7301	9.7120	22.5363	2.1202	0.1149	0.5827
WAN4	Plant Wansley	Coal	0.4457	35.5463	352.0887	1.3503	1.3242	37.5701	487.9978	4.8073	9.5386	18.8229	1.8907	0.1168	0.3823
WAN5	Plant Wansley	Coal	1.0618	88.5689	568.9055	1.8726	3.6784	45.1092	604.1983	5.6414	10.3673	21.6381	2.6759	0.1347	0.6316

Appendix A.2. Continued.

ClamID	Site Name	Site Type	V 51	Mn 55	Fe 57	Co 59	Ni 60	Cu 65	Zn 68	As 75	Se 77	Sr 88	Cd 114	Hg 202	Pb 208
WAN6	Plant Wansley	Coal	0.5995	30.0932	394.9858	0.9669	1.1344	30.6771	434.4060	3.9950	8.1840	17.7822	1.8825	0.1260	0.6876
WAN7	Plant Wansley	Coal	1.4644	56.6625	684.2451	1.3786	1.5019	37.8763	536.0989	5.4825	9.2188	21.1066	2.4069	0.1274	0.8560
WAN9	Plant Wansley	Coal	0.6982	52.3907	471.0573	1.4435	1.7367	43.5620	561.3218	5.4951	10.7776	23.1358	2.5631	0.1453	0.6599
Yates1	Plant Yates	Coal	0.2314	30.7643	257.0384	1.7818	1.4335	72.2232	509.2583	2.9596	11.1417	12.7803	0.1780	0.1276	0.5370
Yates11	Plant Yates	Coal	0.4900	23.2053	402.1983	1.8498	1.3608	136.1979	364.7751	3.8116	9.7371	13.7661	7.2527	0.2413	0.8139
Yates2	Plant Yates	Coal	0.2988	25.9014	263.4188	1.4469	1.4496	58.6375	436.6116	3.0079	12.3009	10.9197	2.2873	0.1265	0.5570
Yates3	Plant Yates	Coal	0.4842	28.1564	413.8707	3.4719	1.8000	99.1555	490.1705	3.8593	11.0924	14.8554	5.1081	0.2406	0.7485
Yates4	Plant Yates	Coal	0.4398	23.0102	425.5482	1.3305	1.1284	93.4604	334.9501	3.5905	18.2522	11.0056	5.6211	0.1576	0.7852
Yates5	Plant Yates	Coal	0.3047	22.9861	270.0425	1.6667	1.4305	78.2883	384.4827	3.9598	14.6926	11.1034	6.7113	0.1539	0.6298
Yates6	Plant Yates	Coal	0.3467	25.6068	300.1501	1.1000	1.2247	61.7439	275.5534	3.2659	13.8615	12.9935	1.3316	0.1307	0.5874
Yates7	Plant Yates	Coal	0.3560	23.1513	336.2350	2.2763	1.3275	102.8104	343.6656	3.6771	15.3352	8.9423	0.2313	0.1578	0.5823
Yates8	Plant Yates	Coal	0.5272	22.8029	386.9463	1.5776	0.8387	87.0468	287.3226	3.3919	15.9443	9.0985	3.8124	0.1630	0.6248
ANW1	Anneewakee	Wastewater	0.2418	20.1861	647.7714	1.5610	1.6574	43.8214	289.3158	2.9516	3.3899	20.4736	2.2615	0.3018	0.8103
ANW10	Anneewakee	Wastewater	0.1679	19.1485	588.9159	2.2971	1.6290	49.1951	288.6841	2.8283	2.7916	27.4686	1.0268	0.2605	0.1952
ANW3	Anneewakee	Wastewater	0.3043	26.0664	638.7752	1.8512	1.7504	35.0943	324.1943	2.6884	3.3368	31.0693	1.2865	0.2017	0.3285
ANW4	Anneewakee	Wastewater	0.2598	35.2571	704.5481	1.1671	1.4128	28.0003	211.7133	2.2931	2.6153	32.6588	1.2014	0.2096	0.2925
ANW5	Anneewakee	Wastewater	0.2414	2.2223	803.7365	0.1791	0.3366	4.0061	28.4230	0.2736	0.3417	2.7798	0.2087	0.0539	0.0338
ANW6	Anneewakee	Wastewater	0.2829	3.0125	609.2553	0.1347	0.1607	3.7939	21.6663	0.3084	0.3149	2.9146	0.1606	0.0223	0.0240
ANW7	Anneewakee	Wastewater	0.2441	27.2705	711.9303	3.3789	2.2050	69.2401	408.0474	3.9208	4.2054	29.8413	2.0637	0.4775	0.4116
ANW8	Anneewakee	Wastewater	0.1773	19.9839	547.1171	1.4898	1.5866	42.5004	274.1806	3.1981	3.2160	21.0797	1.8565	0.3211	0.4408
ANW9	Anneewakee	Wastewater	0.2623	18.3824	682.8819	1.4482	1.6164	43.0198	279.8907	3.0807	3.2682	22.5649	1.5913	0.3190	0.3010
BC1	Big Creek	Wastewater	0.1903	31.1176	404.3262	2.4450	1.0936	39.0676	409.4973	2.5971	3.3457	9.6020	1.5775	0.1045	3.2314
BC10	Big Creek	Wastewater	0.1053	24.8392	309.9874	3.1120	1.8883	42.2679	418.9237	3.0112	3.5096	13.5891	1.3085	0.1191	1.7663
BC12	Big Creek	Wastewater	3.7586	129.7600	3524.8439	3.8065	2.2682	45.0174	361.2819	2.6194	3.5288	15.8378	2.0273	0.2168	2.6407
BC2	Big Creek	Wastewater	0.1587	21.2899	379.5653	2.3836	1.0028	33.4668	372.6189	2.0891	2.8549	9.4207	1.3787	0.0811	2.1499
BC3	Big Creek	Wastewater	0.2509	27.0294	398.3070	2.3506	1.0610	37.9455	355.1807	2.9348	3.5262	9.6892	1.3575	0.1148	3.4274
BC4	Big Creek	Wastewater	0.2339	30.2823	332.6150	2.1289	1.0020	39.7772	375.4823	3.6735	4.1907	11.5776	1.2222	0.1434	1.9601
BC7	Big Creek	Wastewater	0.1950	25.7611	364.3798	1.8886	0.9516	34.4619	372.0482	2.9356	3.8713	8.8725	1.4957	0.1023	2.8755
BC8	Big Creek	Wastewater	0.1694	22.9876	295.7652	1.4397	0.8151	34.3994	261.3612	2.7764	3.6202	7.1177	1.3042	0.0973	2.2186
BC9	Big Creek	Wastewater	0.1237	24.2374	308.0504	1.7352	1.3282	39.0900	315.2169	2.7681	3.1697	9.7782	1.2979	0.1293	1.9331

Appendix A.2. Continued.

ClamID	Site Name	Site Type	V 51	Mn 55	Fe 57	Co 59	Ni 60	Cu 65	Zn 68	As 75	Se 77	Sr 88	Cd 114	Hg 202	Pb 208
SC1	Suwanee Creek	Wastewater	0.1600	23.2298	397.2881	1.3100	0.6554	23.2017	202.7008	1.9439	2.2830	5.9238	1.0522	0.1655	0.3860
SC12	Suwanee Creek	Wastewater	0.2249	28.3190	439.6095	1.7830	0.9456	31.7694	304.2076	2.7483	4.5359	7.6020	1.8727	0.1638	0.2570
SC2	Suwanee Creek	Wastewater	0.2625	24.8670	824.0932	3.8997	1.4776	43.5174	416.7800	3.2451	4.6553	11.5290	1.3460	0.1713	0.4491
SC3	Suwanee Creek	Wastewater	0.2425	21.9898	743.8877	1.7966	0.9874	31.2546	247.6055	2.9542	4.6193	8.0478	1.7084	0.1849	0.3891
SC4	Suwanee Creek	Wastewater	0.2169	23.4033	572.6235	1.2024	0.7506	32.3813	243.6189	3.2981	4.5571	7.0534	1.5791	0.2110	0.2738
SC5	Suwanee Creek	Wastewater	0.2371	23.2594	562.8272	1.6960	0.8310	34.3106	265.7134	2.7608	4.0672	6.9218	1.3545	0.1557	0.5751
SC6	Suwanee Creek	Wastewater	0.2139	26.2328	449.9975	1.5900	0.7601	36.5326	302.6978	2.9711	4.3347	7.4412	1.9547	0.1463	0.3947
SC7	Suwanee Creek	Wastewater	0.2916	32.1963	736.7782	2.6994	2.4638	41.5541	358.1095	4.1494	5.9338	10.4288	2.2624	0.2445	0.4596
SC8	Suwanee Creek	Wastewater	0.2974	31.8595	613.7804	1.9611	1.1327	26.7335	253.3174	2.7309	3.7676	8.7579	1.4824	0.1703	0.3216

Appendix A.3. Dissolved trace element concentrations at all study sites (µg/L) grouped by site type and location.

Site Name	Land use	Element Mass	**V** 51	**Mn** 55	**Fe** 57	**Co** 59	**Ni** 62	**Cu** 63	**Zn** 68	**As** 75	**Se** 77	**Sr** 88	**Cd** 114	**Pb** 208
Broad River	Forest		0.92	13.56	368.48	0.11	0.22	2.13	52.85	0.25	0.34	16.58	BDL	0.20
Flat Shoal Creek	Forest		0.27	336.75	670.81	0.24	0.17	0.57	30.07	0.19	0.19	26.79	BDL	0.07
Snake Creek	Forest		0.19	27.95	372.31	0.08	0.16	0.74	29.64	0.21	0.20	11.37	0.01	0.06
East Fork Little River	Agriculture		0.20	19.96	147.11	0.12	BDL	1.69	22.16	0.12	0.11	28.73	0.01	0.07
Wahoo Creek	Agriculture		0.66	28.83	223.52	0.08	BDL	0.65	27.96	0.18	0.20	48.92	BDL	0.04
West Fork Little River	Agriculture		0.29	15.32	146.87	0.10	BDL	0.67	32.68	0.13	0.18	44.07	0.01	0.02
Nancy Creek	Urban		0.54	108.17	352.97	0.26	0.41	5.08	65.89	0.74	0.37	51.55	0.02	0.29
Peachtree Creek	Urban		0.22	66.49	385.27	0.21	0.11	2.52	38.72	0.41	0.48	69.74	0.03	0.17
Sope Creek	Urban		0.09	109.64	270.20	0.36	BDL	0.96	32.96	0.30	0.24	50.70	0.02	0.02
Plant McDonough	Coal		0.38	44.02	169.77	0.32	0.86	2.62	32.61	0.36	0.41	39.57	0.05	0.15
Plant Wansley	Coal		67.51	39.54	876.42	1.34	6.71	3.18	30.78	81.09	29.42	1299.14	1.66	0.11
Plant Yates	Coal		2.06	55.69	286.80	0.62	2.09	3.12	48.75	1.50	3.53	97.91	0.12	0.20
Anneewakee Creek	Wastewater		0.30	20.56	357.56	0.18	0.34	1.56	31.15	0.34	0.24	20.47	0.01	0.23
Big Creek	Wastewater		0.21	93.41	416.70	0.21	0.01	1.00	24.40	0.21	0.39	37.67	0.01	0.05
Suwanee Creek	Wastewater		0.30	251.90	779.94	1.04	0.69	2.03	34.86	0.39	0.30	39.07	0.04	2.21

Appendix A.4. Mean clam lengths with standard errors from all fifteen study sites (N=9).

Site Name	Site Type	Shell Length
Broad River	Forest	17.21 ± 0.27
Flat Shoals Creek	Forest	19.30 ± 0.17
Snake Creek	Forest	17.90 ± 0.43
East Fork Little River	Agriculture	17.03 ± 0.30
Wahoo Creek	Agriculture	18.68 ± 0.27
West Fork Little River	Agriculture	18.18 ± 0.20
Nancy Creek	Urban	18.80 ± 0.24
Peachtree Creek	Urban	18.12 ± 0.23
Sope Creek	Urban	16.81 ± 0.28
Plant McDonough	Coal	20.50 ± 0.80
Plant Wansley	Coal	17.42 ± 0.42
Plant Yates	Coal	17.89 ± 0.32
Anneewakee Creek	Wastewater	19.77 ± 0.43
Big Creek	Wastewater	19.42 ± 0.57
Suwanee Creek	Wastewater	18.91 ± 0.41

APPENDIX B

SUPPLEMENTAL MATERIAL TO CHAPTER 3

Appendix B.1. Dissolved concentrations of trace elements (µg/L) grouped by site and timepoint.

Site	Day	V 51	Mn 55	Fe 55	Co 59	Ni 62	Cu 63	Zn 66	As 75	Se 77	Sr 88	Cd 111	Pb 208
Meyers Branch	0	0.3658	22.6787	524.1717	0.1277	0.0000	0.4854	29.7176	0.1732	0.2165	22.4805	0.0000	0.0840
Meyers Branch	28	0.4367	29.0166	456.8244	0.1598	0.0414	0.5837	30.7903	0.1637	0.4057	23.6649	0.0000	0.0941
Meyers Branch	56	0.3235	14.9709	373.9242	0.1073	0.1027	0.2392	25.9132	0.1818	0.2398	23.0568	0.0000	0.0914
Meyers Branch	84	0.2671	12.7683	355.4746	0.0370	0.0823	0.0975	21.4249	0.3368	0.2222	20.9439	0.0835	0.0196
Beaver Dam Creek (A)	0	7.4169	22.9731	80.2105	0.2853	3.6337	3.6102	29.3627	12.5034	2.3922	107.2479	0.1834	0.0056
Beaver Dam Creek (A)	28	7.1596	9.9317	93.2660	0.1088	2.7040	2.5921	13.9273	16.2459	2.6849	140.1163	0.1597	0.0000
Beaver Dam Creek (A)	56	9.0813	11.4116	98.7208	0.1250	1.7115	1.7808	12.6973	17.2989	2.8446	208.6984	0.0619	0.0796
Beaver Dam Creek (A)	84	9.6420	9.6926	121.9330	0.0847	2.6667	2.1313	18.8875	27.2006	3.1073	291.0037	0.1077	0.0145
Beaver Dam Creek (B)	0	1.1867	31.1280	167.0793	0.2598	0.6038	1.8479	37.0094	1.0541	0.4033	36.1624	0.0360	0.1203
Beaver Dam Creek (B)	28	1.3051	37.6714	190.8639	0.3747	0.8697	1.7193	27.8978	1.8966	0.6439	41.2517	0.0341	0.0479
Beaver Dam Creek (B)	56	1.1177	35.7378	146.5618	0.1341	0.2826	2.5895	26.6044	1.2891	0.4192	39.8833	0.0000	0.0333
Beaver Dam Creek (B)	84	1.5643	30.8655	185.9251	0.1334	0.5747	1.4718	22.4552	3.0076	0.7713	53.9878	0.0528	0.0879
Beaver Dam Creek (C)	0	1.3125	32.0144	210.2968	0.2491	0.5646	2.4275	40.0624	1.1457	0.7513	43.5158	0.0439	0.1344
Beaver Dam Creek (C)	28	1.2175	35.0923	177.3870	0.3622	1.0282	1.8826	28.1562	1.8036	0.5709	41.1684	0.0196	0.0441
Beaver Dam Creek (C)	56	1.1576	23.7684	197.8980	0.1127	0.2640	1.7284	25.6858	1.2486	0.3937	40.6231	0.0025	0.2090
Beaver Dam Creek (C)	84	1.3390	21.1874	174.7358	0.0935	0.4769	1.3103	22.2984	2.5792	0.5575	49.5941	0.0851	0.0917
Beaver Dam Creek (D)	0	1.1240	27.5479	158.5399	0.1937	0.4476	1.7726	26.6541	0.9827	0.3994	35.8356	0.0179	0.0571
Beaver Dam Creek (D)	28	1.2354	38.0866	181.4017	0.2952	1.0138	1.5240	28.5623	1.8820	0.5219	43.8807	0.0208	0.0556
Beaver Dam Creek (D)	56	0.9787	19.0786	132.2376	0.0904	0.2392	1.2949	25.7715	1.0742	0.3106	35.7538	0.0062	0.0479
Beaver Dam Creek (D)	84	1.3653	20.5048	180.6819	0.0921	0.6008	1.4840	22.9407	2.7140	0.7969	53.3696	0.0963	0.0918

Appendix B.2. Tissue concentrations (μg/g dry mass) of resident *Corbicula fluminea* at the beginning of the experiment grouped by site.

	Element	**V**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Sr**	**Cd**	**Hg**	**Pb**
	Mass	**51**	**55**	**57**	**59**	**60**	**63**	**68**	**75**	**77**	**88**	**114**	**200**	**208**
ClamID	**Location**													
A-0-1	Site A	0.5742	20.3967	503.5628	3.8976	2.2641	99.3156	188.5137	4.0699	6.3523	15.0413	30.7098	0.1240	0.2591
A-0-2	Site A	0.9739	13.6421	275.7951	3.3573	7.2233	334.7941	196.5883	8.3622	24.2049	20.0175	35.9007	0.1292	0.8294
A-0-3	Site A	0.7792	10.6378	207.9472	3.5124	5.5324	312.1969	115.8078	8.1789	23.1854	6.7384	44.4447	0.1354	1.4258
A-0-4	Site A	1.4035	10.6701	397.7625	4.0838	5.7714	1606.8242	187.9340	6.8738	23.6623	29.6923	57.8363	0.4088	1.1154
A-0-5	Site A	0.5699	8.0258	159.6949	2.9047	5.6577	308.4187	148.3991	6.1517	16.9157	11.2631	35.1953	0.1081	0.5494
A-0-6	Site A	0.6089	8.3210	155.5266	2.3146	5.1490	416.5609	182.3028	7.2985	20.1408	13.5855	25.4604	0.1152	0.9702
A-0-7	Site A	0.6159	9.4418	179.2483	2.8094	5.8170	332.5984	116.0108	7.0320	19.5177	13.1330	37.7842	0.0939	0.5704
A-0-8	Site A	0.7357	10.0649	191.9846	3.6600	6.9611	318.8866	198.7848	8.0549	23.7288	16.5987	46.6572	0.1295	0.8791
B-0-1	Site B	0.3130	18.7213	420.5656	1.5275	1.3659	84.3461	307.2790	5.0316	7.7724	14.1137	3.7785	0.3011	0.7860
B-0-2	Site B	0.4094	15.6077	690.4789	2.6955	1.8448	124.4573	183.5516	6.0453	9.9323	15.0012	5.7967	0.6365	0.4944
B-0-3	Site B	0.3890	11.7249	425.5316	2.1770	1.6813	65.6920	245.8183	3.4869	7.4510	10.4094	3.9688	0.2701	0.3543
B-0-4	Site B	0.7064	66.8643	759.8089	2.9572	2.0965	53.9495	216.2335	3.0802	6.4847	9.0638	3.3290	0.0974	0.3914
B-0-5	Site B	0.6089	66.6558	440.5355	3.2875	2.3546	77.4959	236.9903	4.0964	9.1066	9.8918	5.1731	0.2277	0.6977
B-0-6	Site B	0.4055	29.8969	419.1454	2.0425	1.6955	72.0844	194.6019	4.1498	7.2869	9.2973	4.1347	0.3657	0.2988
B-0-7	Site B	0.3376	27.3327	444.7609	2.2490	1.4211	98.2236	184.0274	5.0961	7.1768	8.9491	5.2837	0.5082	2.4514
B-0-8	Site B	0.3968	16.4707	395.2059	1.4268	1.2477	79.9469	168.5397	4.5642	6.8439	11.6942	3.3791	0.4927	0.7176

Appendix B.2. Continued.

	Element	V	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	Hg	Pb
	Mass	51	55	57	59	60	63	68	75	77	88	114	200	208
ClamID	**Location**													
C-0-1	Site C	0.3727	17.9529	472.2712	3.5088	2.0858	96.7549	206.6650	5.4296	9.7161	10.6427	3.9285	0.4414	0.2375
C-0-2	Site C	0.6560	33.7959	503.0001	3.7910	2.9994	77.9888	225.7046	5.3751	8.3625	10.1245	3.5501	0.3784	0.4724
C-0-3	Site C	0.5707	24.7730	494.2884	4.0837	3.0439	74.7171	260.4219	4.9041	8.6522	10.7260	3.7024	0.2593	1.4458
C-0-4	Site C	0.7031	34.1013	599.5158	4.5020	2.9792	84.9094	252.0884	5.2845	9.2873	12.6820	4.1701	0.3483	0.4142
C-0-5	Site C	0.5689	27.5327	590.6474	3.5289	2.6197	97.8056	201.5608	5.8521	9.4496	13.1405	4.1507	0.4962	0.3107
C-0-6	Site C	0.5345	25.1234	458.0859	3.8035	2.5522	90.1354	235.4359	5.7144	9.4497	15.5525	4.1533	0.4289	0.4535
C-0-7	Site C	0.3284	15.8159	271.0574	2.8320	1.9263	60.5798	156.0662	3.4507	6.5182	7.2284	3.1376	0.2053	0.1499
C-0-8	Site C	0.5007	18.2552	453.1227	3.6284	2.4724	81.1916	204.8723	4.7821	9.0507	11.0874	4.3322	0.2790	0.2986
D-0-1	Site D	0.3528	22.5715	439.5190	4.1252	3.5131	84.8534	274.9792	5.5879	8.8373	12.4605	3.4489	0.3981	0.2596
D-0-2	Site D	0.4071	31.2062	626.3543	3.4890	3.2280	126.2110	249.5436	7.7510	9.8885	14.0584	4.8205	0.8075	1.8894
D-0-3	Site D	0.8403	115.9193	644.5903	2.7503	3.4335	60.3396	247.3488	3.8041	7.2234	11.3582	2.8003	0.4503	1.1660
D-0-4	Site D	0.2651	69.6097	947.3617	38.8083	37.4734	97.0837	325.1986	43.8756	44.3782	45.2656	37.2840	0.4302	43.7760
D-0-5	Site D	0.4728	31.0460	457.7542	2.4558	2.6092	50.0915	233.8311	3.4296	6.1878	11.0151	3.2655	0.1702	1.0509
D-0-6	Site D	0.5088	25.9478	636.6663	8.9870	4.6132	178.1718	346.9692	6.7180	10.4857	15.4715	3.4311	0.7390	0.2871
D-0-7	Site D	0.4082	27.7701	509.1633	2.9596	2.3220	113.6821	221.4875	5.8797	9.1588	12.0026	2.7130	0.7015	1.1443
D-0-8	Site D	0.7840	46.4186	1156.9387	13.1659	9.3170	312.6976	571.0977	12.5293	21.3444	48.0100	8.2671	1.6345	0.5653
M-0-1	Meyers	0.2385	48.5613	863.5559	4.2753	1.7871	76.2923	169.4153	4.3038	4.2163	8.2444	1.3646	7.3130	0.5732
M-0-2	Meyers	0.3497	30.6455	719.2963	2.8060	1.8134	37.4160	164.2200	2.7687	4.3726	6.1193	1.1106	1.6397	0.3262
M-0-3	Meyers	0.7919	165.9977	1106.9457	3.0265	1.6180	52.5485	187.1135	3.8661	4.3073	14.0314	1.3559	2.7512	0.9148
M-0-4	Meyers	0.2401	48.6789	1106.0960	5.8979	1.9902	129.4979	178.4483	5.1765	5.2282	7.3148	1.8572	8.4261	0.5919
M-0-5	Meyers	0.3844	46.3372	893.9041	4.9290	2.3756	71.5754	218.9889	4.4203	4.2940	27.1894	1.2886	6.2648	1.0866
M-0-6	Meyers	0.3292	35.4584	924.8813	3.3033	1.7239	53.6472	177.3551	3.5041	4.8759	6.2435	1.2933	3.3787	0.9094
M-0-7	Meyers	0.2527	70.7378	1025.7505	4.3346	2.1079	82.7370	169.5807	4.3852	4.8165	8.8542	1.4305	4.8524	0.6267
M-0-8	Meyers	0.3506	34.2816	824.3863	2.6417	1.8445	40.8079	146.1414	2.9992	3.9725	6.0148	1.1864	2.4291	0.4773
M-0-9	Meyers	1.4295	45.9818	947.1108	2.3962	2.6486	41.5312	251.1678	3.3407	4.0313	82.5751	0.9175	1.9578	4.2240

Appendix B.3. Tissue concentrations (µg/g dry mass) of transplanted *Corbicula fluminea* grouped by site and timepoint.

			Element	V	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	Hg	Pb
			Mass	51	55	57	59	60	63	68	75	77	88	114	200	208
Sample ID	**Location**	**Timepoint**	**Cage**													
A-1-1	Site A	1	1	1.4185	89.0627	603.9770	5.3722	17.9243	153.8398	264.8255	7.6211	15.1571	17.2498	24.2079	2.3413	0.9964
A-1-2	Site A	1	1	1.8982	121.8932	686.0369	5.6608	18.6357	126.8430	276.8336	8.9479	17.3557	15.8371	23.3096	2.0589	1.1247
A-1-3	Site A	1	2	1.6807	104.3223	627.2777	5.8718	18.7576	116.4849	282.8892	7.6577	15.7896	17.6464	24.8300	2.2081	1.0876
A-1-4	Site A	1	2	1.5940	104.0359	558.5405	4.3762	14.7799	93.3051	233.4735	7.7908	14.8410	16.3476	22.3532	1.5662	0.9059
A-1-5	Site A	1	3	1.9870	235.1844	800.0231	6.3834	20.4593	118.4188	288.1468	9.1604	16.7302	18.8947	29.0420	1.9823	1.2009
A-1-6	Site A	1	3	1.2716	70.4200	585.4117	4.5337	17.0417	126.6550	269.0541	7.0831	16.0890	20.1277	23.3006	2.6714	0.8311
A-1-7	Site A	1	4	1.3741	76.6716	491.0205	4.2971	16.0275	105.7248	235.5988	7.3052	15.2004	13.5185	25.6628	1.7639	0.8947
A-1-8	Site A	1	4	1.1563	65.6062	581.9000	4.5144	14.4674	109.1383	252.4475	7.8733	16.9690	16.3219	24.6712	2.0687	1.0826
A-2-1	Site A	2	1	0.6559	10.9968	269.1646	2.2514	7.6912	74.8190	224.8028	10.0068	26.4497	9.6605	14.7731	0.6265	0.3330
A-2-2	Site A	2	1	0.4730	8.4724	228.8433	2.0637	8.2164	68.1024	165.3651	7.4943	17.6964	8.8283	15.0343	0.8711	0.3161
A-2-3	Site A	2	2	0.4569	7.9766	236.0162	1.3198	4.4310	47.7723	150.5257	7.1020	16.7570	7.8761	11.9774	0.9582	0.2067
A-2-4	Site A	2	2	0.5870	8.1543	236.2397	1.2932	3.5927	52.8022	129.2692	7.5456	16.0041	9.7337	12.9909	0.9454	0.2781
A-2-5	Site A	2	3	0.7573	12.2767	147.4906	1.7156	7.2664	56.6239	220.0877	7.4338	19.5689	9.3302	14.4327	0.6764	0.4183
A-2-6	Site A	2	3	0.6941	5.8893	231.1749	1.4496	6.5316	64.6097	144.0306	6.8133	17.7941	5.2597	17.4881	0.8534	0.3270
A-2-7	Site A	2	4	0.5988	13.0459	256.9333	1.6828	6.4574	52.1180	168.9395	7.7717	18.3851	11.4267	14.8810	0.8794	0.2988
A-2-8	Site A	2	4	0.6723	15.0394	285.2943	2.0194	10.0503	67.9570	238.9491	9.4535	22.3694	14.1272	16.2201	0.8268	3.2038
A-3-1	Site A	3	1	0.3884	7.2253	171.6124	1.5471	7.5203	55.5592	174.6073	9.0609	20.1154	10.9121	7.9146	0.5190	0.3153
A-3-2	Site A	3	1	0.6188	13.9015	175.0801	2.1465	11.1518	66.0784	188.6666	8.4551	19.2420	35.8492	9.1157	0.6185	0.3426
A-3-3	Site A	3	1	0.3598	7.0173	90.7769	1.2462	5.0604	45.9733	174.8839	7.7719	14.6805	9.0074	5.3581	0.4334	0.2625
A-3-4	Site A	3	2	0.4255	5.5690	178.8680	1.3848	4.7057	61.0072	156.0279	6.9764	14.9507	6.6845	9.9157	1.1500	0.1839
A-3-5	Site A	3	2	0.4489	9.5698	161.1881	1.3782	6.3200	57.5582	181.0279	10.2267	20.6777	10.3756	7.8339	0.5525	0.3175
A-3-6	Site A	3	2	0.4084	9.0178	108.8464	1.1636	4.4276	59.0728	138.0060	7.8725	17.1146	6.7550	7.1444	0.9403	0.2708
A-3-7	Site A	3	3	0.4263	7.0860	170.6405	1.0247	3.8117	48.0946	173.0619	7.3504	15.1913	9.1560	6.4981	0.7124	0.1771
A-3-8	Site A	3	3	0.5553	12.7741	160.7599	1.2867	6.2263	43.0387	144.9291	7.3625	15.1299	22.9192	8.1382	0.3517	0.2762
A-3-9	Site A	3	3	0.5047	14.0880	118.3507	1.3696	7.0587	50.7542	181.1099	7.6816	17.3848	23.3442	7.2393	0.4105	0.3160
A-3-10	Site A	3	4	0.2835	8.0521	208.2231	1.7541	6.6211	68.9451	163.9167	9.6128	22.9059	7.0099	9.9591	0.8687	0.3317
A-3-11	Site A	3	4	0.5279	13.6329	173.4653	1.0208	4.2161	47.2119	131.4095	9.0935	20.0036	10.3801	7.8792	0.2983	0.3129
A-3-12	Site A	3	4	0.4349	7.3071	91.5844	1.1044	5.1634	46.2401	136.1851	7.3356	15.6328	5.5112	6.3977	0.4753	0.2579

Appendix B.3. Continued.

				Element	V	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	Hg	Pb
				Mass	51	55	57	59	60	63	68	75	77	88	114	200	208
Sample ID	Location	Timepoint	Cage														
B-1-1	Site B	1	1		0.1596	17.3931	601.0113	3.7634	4.3955	68.5678	195.4237	3.7645	6.1238	11.1869	2.2275	2.7490	1.1994
B-1-2	Site B	1	1		0.5087	37.3851	873.1131	4.8796	5.1310	88.6290	223.2086	4.3642	6.1454	19.1794	2.0195	4.9250	1.1998
B-1-3	Site B	1	2		0.0985	35.3374	1232.3022	3.4509	6.1240	64.6884	137.3967	3.1610	4.4514	95.6085	1.6663	3.0953	0.8250
B-1-4	Site B	1	2		0.0633	16.4213	750.6406	4.7060	5.2713	83.3051	221.2358	4.7436	5.7894	16.1389	2.2383	6.2103	3.2090
B-1-5	Site B	1	3		0.2312	35.8373	683.3491	3.2635	3.2410	79.4060	199.4421	4.1906	6.9839	13.0660	2.3659	5.3946	0.4462
B-1-6	Site B	1	3		0.5421	173.9195	1070.5484	5.2602	4.8805	79.3404	178.3188	3.9337	6.2806	16.4044	2.4468	4.1685	0.5450
B-1-7	Site B	1	4		0.0911	23.8728	719.5149	5.7426	5.5307	93.6240	258.1164	4.4915	6.3242	22.0037	2.3950	4.0254	1.0186
B-1-8	Site B	1	4		0.2638	42.1026	727.8521	3.4981	4.3225	66.8336	274.7240	3.5650	5.7480	22.6573	1.8577	3.9425	0.6065
B-2-1	Site B	2	1		0.1538	70.0520	362.1406	2.8089	4.9902	103.2699	194.8344	5.6595	8.6052	92.1629	3.3011	3.4275	0.3334
B-2-2	Site B	2	1		0.3717	62.8437	722.7723	2.5520	2.0700	96.2638	207.7595	5.1443	9.1872	10.5737	2.7608	2.8471	1.3989
B-2-3	Site B	2	2		0.4278	39.0809	981.2543	1.9216	1.4643	65.7727	174.1844	4.7782	8.6569	8.4554	4.5798	3.0537	0.5183
B-2-4	Site B	2	2		0.5945	172.6411	1054.9507	2.6587	2.0463	99.7110	176.3031	4.9080	8.0496	8.6476	4.9991	4.3484	0.4598
B-2-5	Site B	2	3		0.1317	95.0348	733.0349	2.6303	1.5631	102.1390	179.7572	4.9995	8.5692	5.5505	3.0975	5.4504	1.3866
B-2-6	Site B	2	3		0.3274	90.3274	803.1751	1.9901	2.1052	68.4973	151.7213	4.1435	7.2951	28.2722	3.7352	3.5131	0.3227
B-2-7	Site B	2	4		0.2506	25.2106	830.4794	1.9816	1.1398	69.9500	192.3322	4.8193	8.9935	14.1717	5.2289	1.9974	0.2378
B-2-8	Site B	2	4		0.4392	39.9263	637.2437	2.1700	1.7104	59.0415	205.3484	4.3568	8.8854	-3.1594	5.1624	0.7751	0.4145
B-3-1	Site B	3	1		0.2057	18.8027	593.8701	2.4134	2.0240	91.3628	212.4728	5.2995	9.3574	2.8998	3.9601	1.5960	0.2596
B-3-2	Site B	3	1		0.2867	226.6917	761.0672	3.3121	1.7441	119.2404	171.6207	5.5489	9.0667	9.8343	2.3058	6.4910	0.3015
B-3-3	Site B	3	2		0.2266	37.3255	662.9901	2.0367	1.5601	68.0269	200.8439	5.7715	9.5566	5.7874	3.4309	2.0584	0.3753
B-3-4	Site B	3	2		0.1798	42.7708	598.8957	1.9046	1.6700	87.0177	182.2184	5.1603	8.4020	5.4669	2.4944	2.6248	0.2538
B-3-5	Site B	3	3		0.1413	24.0103	640.4366	2.0896	1.5667	76.6841	181.6710	5.1144	7.8612	1.5904	2.4035	1.6589	0.1926
B-3-6	Site B	3	3		0.1600	42.8283	669.6000	2.6012	1.8605	105.0969	180.2069	6.1091	8.6298	15.5198	2.6430	4.2323	0.2835
B-3-9	Site B	3	3		0.0901	40.3559	944.9042	2.5631	1.5584	129.3095	170.2675	6.3693	9.3200	6.2631	2.4605	4.7852	0.3390
B-3-7	Site B	3	4		0.3027	100.9732	670.0605	2.8240	1.9218	115.7282	186.0824	6.0665	9.8495	7.7311	2.8041	4.3552	0.3673
B-3-8	Site B	3	4		0.2432	28.7965	626.1661	1.9619	1.6094	76.0279	186.9294	5.3969	8.9962	4.6436	2.8396	1.4797	0.3097
B-3-11	Site B	3	4		0.2316	27.5243	371.2836	1.6960	1.5944	56.9453	180.6211	4.1133	7.6060	8.0705	2.3305	1.1433	0.2426

Appendix B.3. Continued.

				Element	V	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	Hg	Pb
				Mass	51	55	57	59	60	63	68	75	77	88	114	200	208
Sample ID	Location	Timepoint	Cage														
C-1-1	Site C	1	1		0.4645	51.5886	979.0370	3.5790	4.4763	64.3897	203.4677	4.0349	5.9085	51.5544	2.1401	3.7750	0.4142
C-1-2	Site C	1	1		0.3657	18.0658	759.8341	4.0719	4.0550	75.0476	207.4136	4.0678	6.2625	13.6273	1.8805	4.8878	0.6238
C-1-3	Site C	1	2		0.5336	59.6822	997.0226	4.5936	4.9822	62.0594	253.2126	3.8708	6.4134	18.5172	2.1255	2.6943	0.8559
C-1-4	Site C	1	2		0.3397	34.1720	868.2203	4.2866	4.1553	77.6137	234.8146	4.4636	6.8098	16.2633	1.9648	6.1983	1.0699
C-1-5	Site C	1	3		0.4726	31.3459	887.8706	2.4685	2.5570	63.2519	184.6078	3.7117	6.3975	9.7979	2.0103	3.6611	0.6563
C-1-6	Site C	1	3		0.5996	38.2334	889.2826	4.0272	4.3945	64.9950	261.3706	4.1988	7.4439	13.4860	2.1049	1.8553	0.4903
C-1-7	Site C	1	4		0.2699	28.6179	1205.2306	3.2614	4.9042	78.9682	171.8731	3.7509	6.0314	102.0103	1.7786	5.2643	0.5683
C-1-8	Site C	1	4		0.4317	27.8910	930.1853	4.6254	5.3195	64.8686	251.3642	3.7389	5.7974	12.4943	1.8114	3.3852	0.6819
C-2-1	Site C	2	1		0.3223	48.8136	1170.0119	2.8263	1.7839	73.1937	245.2262	6.1325	10.4902	12.2451	4.8472	3.3323	0.3274
C-2-2	Site C	2	1		6.6704	351.5313	4125.1296	5.0927	6.3815	89.7923	199.8555	5.9506	8.6807	18.6743	5.3172	3.0641	2.3822
C-2-3	Site C	2	2		0.1722	16.3695	666.2029	2.0317	1.6212	65.8231	173.1069	4.2134	7.6568	7.9793	5.0290	2.8877	0.3803
C-2-4	Site C	2	2		0.8577	57.0705	871.6057	2.4258	2.4211	66.7076	220.7026	4.7369	8.7938	-0.7989	5.6773	1.6452	0.5250
C-2-5	Site C	2	3		0.3625	34.8925	759.4472	2.8943	2.0062	83.0848	182.4095	4.4530	7.5903	12.4292	2.2327	3.2235	0.4900
C-2-6	Site C	2	3		0.3214	37.2149	649.9921	1.5315	1.5134	41.3605	172.0718	4.1830	7.7658	7.6323	1.7416	1.1781	0.2770
C-2-7	Site C	2	4		0.8295	70.5102	940.7683	1.9828	1.9843	69.3527	185.5181	4.5542	7.5009	6.4802	2.9493	2.6298	2.1530
C-2-8	Site C	2	4		0.5640	41.5617	1036.0581	2.6405	1.6730	93.4588	194.5476	5.4813	8.6560	8.3992	4.6568	4.0029	0.5083
C-3-1	Site C	3	1		0.3855	50.4057	800.8063	2.0987	1.8722	77.1110	216.1901	5.5578	9.1932	8.6999	2.4507	2.7560	0.3701
C-3-2	Site C	3	1		0.5020	41.9545	798.8442	1.9350	2.2785	71.8159	193.6512	5.1752	8.9966	1.7005	2.5596	1.8933	0.3260
C-3-3	Site C	3	2		0.3000	49.1293	679.7874	1.7078	2.0671	57.0265	170.5505	4.8638	7.0992	12.6588	2.7964	1.7832	0.4538
C-3-4	Site C	3	2		0.4972	50.6114	857.7047	2.1064	2.0803	79.6913	194.9076	6.1756	8.9813	12.0880	2.8827	2.7024	0.5087
C-3-5	Site C	3	3		0.7401	32.1843	958.3203	2.3147	1.0870	36.8420	201.6738	5.6931	8.8269	4.2895	2.6376	1.9996	0.2471
C-3-6	Site C	3	3		0.2374	19.8872	987.3032	2.7185	2.9078	71.7721	207.8986	5.3106	8.2997	44.9520	2.8335	1.9350	0.4134
C-3-7	Site C	3	4		0.4429	35.4368	834.4298	2.1345	1.9752	82.4866	199.9403	5.9404	8.9453	6.3373	3.1164	4.6162	0.5464
C-3-8	Site C	3	4		0.2123	22.0703	815.9827	3.0278	1.8331	78.7784	204.8828	4.0827	7.2093	2.6164	2.8976	3.4190	0.2823
C-3-9	Site C	3	2		0.3811	32.3289	533.4065	2.3633	1.8951	78.3461	187.8638	4.3416	7.6106	4.6703	2.3238	3.6612	0.4864

Appendix B.3. Continued.

				Element	V	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	Hg	Pb
				Mass	51	55	57	59	60	63	68	75	77	88	114	200	208
Sample ID	**Location**	**Timepoint**	**Cage**														
D-1-1	Site D	1	1		0.2453	13.5423	742.9728	4.2277	3.1717	69.5038	201.5554	3.8144	6.8517	9.1520	2.4125	3.7091	0.4443
D-1-2	Site D	1	1		0.4551	17.5160	888.8150	2.9194	6.6611	65.5869	181.9544	4.4078	6.9938	9.3769	2.3095	3.4017	1.0280
D-1-3	Site D	1	2		0.4091	17.7273	820.9259	3.1934	3.4026	71.3106	160.2279	3.8939	6.0394	9.1680	2.3801	4.5020	1.3894
D-1-4	Site D	1	2		0.2811	11.8554	921.5209	4.2280	4.5807	90.2440	198.1046	4.3237	6.6536	14.5766	2.1213	5.8359	0.7801
D-1-5	Site D	1	3		0.1877	13.2234	729.5449	5.4254	5.1721	80.8375	246.0739	3.9968	6.7719	12.9224	2.2900	2.6669	0.6571
D-1-6	Site D	1	3		0.2589	19.1206	729.2729	3.2289	3.0869	67.5508	166.1980	3.8400	6.0259	8.5030	2.1472	3.1627	0.4626
D-1-7	Site D	1	4		0.2073	13.2947	761.2138	3.6073	3.5291	65.2450	185.2336	3.6350	5.7569	10.9238	2.0468	3.4423	0.5398
D-1-8	Site D	1	4		0.3893	14.6746	848.0170	3.1280	3.6039	77.8732	167.5525	4.1296	6.0967	12.1065	2.0668	4.4509	1.3123
D-2-1	Site D	2	1		0.4677	39.1404	749.6839	2.2849	1.7577	71.2666	211.9273	4.4413	7.7259	15.0716	4.9108	1.8625	0.5132
D-2-2	Site D	2	1		0.4715	31.0263	818.7784	2.5958	2.2789	88.5353	206.2416	4.5365	8.0844	10.3202	5.6676	3.0208	0.7766
D-2-3	Site D	2	2		0.4105	26.7476	936.4785	2.5828	1.8146	104.1525	192.0071	5.6518	8.3876	10.4941	3.0329	4.6213	0.4715
D-2-4	Site D	2	2		0.5885	21.7629	879.8802	2.3935	1.4786	81.9647	187.1803	5.0829	8.4572	8.4829	5.1740	4.6568	0.5334
D-2-5	Site D	2	3		0.4738	72.3967	976.3334	2.7158	1.9870	90.5068	174.3274	4.3521	7.3715	0.1601	4.9613	2.5683	1.4191
D-2-6	Site D	2	3		0.5730	51.9267	806.8319	2.0211	1.6581	79.1638	183.4388	4.2995	7.8955	4.9326	2.7294	2.6886	1.5094
D-2-7	Site D	2	4		0.4761	33.3346	979.2717	2.5466	1.6310	72.5545	177.0652	5.0588	9.0493	12.3693	6.5443	3.2356	0.4410
D-3-1	Site D	3	1		0.2585	16.2763	811.0421	2.4123	1.6975	99.8030	185.4431	5.3881	8.9260	17.0975	1.8913	3.5757	0.2883
D-3-2	Site D	3	1		0.2712	22.5089	660.4291	1.9416	1.5393	69.0111	183.6512	5.0276	8.0396	7.8318	2.2887	4.1614	0.5288
D-3-3	Site D	3	2		0.5179	22.5473	1079.8742	2.5526	2.6449	105.5195	219.2560	7.1484	10.5691	10.7163	3.6186	5.5970	0.8355
D-3-4	Site D	3	2		0.3452	14.4476	805.0036	2.4204	1.9493	86.9344	198.9379	5.4224	8.9030	8.6085	2.4539	3.3036	0.3403
D-3-5	Site D	3	3		0.2262	25.0496	751.5281	1.9170	1.9243	69.9217	188.6757	5.1931	8.2373	13.9021	3.0522	2.7863	0.3869
D-3-6	Site D	3	3		0.2295	36.0779	897.6233	2.5110	2.1007	77.0982	193.4293	5.5680	10.2850	4.2033	3.4397	3.7923	0.5618
D-3-7	Site D	3	3		0.2882	47.0424	798.3798	2.4856	2.3809	76.0583	187.4496	5.1151	9.3802	10.9697	3.0507	2.3752	0.5284
D-3-8	Site D	3	3		0.3044	59.0616	785.3813	2.3734	1.6856	84.4430	195.2317	6.4506	9.7937	4.4472	2.6845	2.9642	0.5239

Appendix B.3. Continued.

				Element	V	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	Hg	Pb
				Mass	51	55	57	59	60	63	68	75	77	88	114	200	208
Sample ID	**Location**	**Timepoint**	**Cage**														
M-1-1	Meyers	1	1		0.3878	30.0837	839.5936	3.0147	1.5377	55.7369	215.6596	3.1718	4.1931	6.3806	1.2244	4.6468	0.6062
M-1-2	Meyers	1	1		0.1939	23.8912	823.1351	3.1516	1.6891	56.1504	171.1076	3.3990	4.2102	6.3120	1.5731	2.6790	0.4096
M-1-3	Meyers	1	2		0.3404	49.0805	938.5411	3.0065	1.8157	61.7937	161.6042	3.3208	4.2877	5.7823	1.7221	3.3923	0.4340
M-1-4	Meyers	1	2		0.1196	19.0312	994.9644	3.3495	2.9964	59.5931	161.4556	3.4521	4.3109	40.7761	1.7300	4.2751	0.3194
M-1-5	Meyers	1	3		0.2529	33.2863	853.0004	3.4652	2.2100	72.4179	159.1299	3.7059	4.6275	6.3368	1.6235	4.6317	0.4387
M-1-6	Meyers	1	3		0.2333	22.3987	810.8331	3.2889	2.0016	57.7609	188.3720	3.5729	4.1530	8.7611	1.7342	3.9905	1.1681
M-1-7	Meyers	1	4		0.2486	31.4622	813.4994	3.6366	1.5882	87.4269	140.2218	3.7060	4.1548	6.0502	1.4931	6.0451	0.4271
M-1-8	Meyers	1	4		0.1492	20.9347	651.4155	2.2830	1.7180	43.2923	139.5043	2.9726	3.2462	6.2846	1.3985	2.9835	1.1898
M-2-1	Meyers	2	1		0.6693	125.4531	1449.4197	4.2806	2.7678	88.3346	214.3770	3.7308	4.3431	16.2233	7.5741	3.7208	1.0981
M-2-2	Meyers	2	1		1.9112	264.0441	2079.7576	5.0679	2.4885	104.6097	200.7835	4.8001	4.3481	13.7398	1.5261	8.3556	1.9996
M-2-3	Meyers	2	2		1.4938	251.0887	1995.2871	3.9625	2.9799	62.7162	240.0167	4.1622	4.5269	9.9522	1.4516	2.7400	2.8616
M-2-4	Meyers	2	2		1.8241	254.1237	2139.2264	4.5618	1.9930	69.9360	235.3463	3.7213	4.1974	11.3077	4.9514	4.8547	1.6103
M-2-5	Meyers	2	3		1.8149	200.3484	1625.7231	3.1501	2.7707	75.5457	141.0545	3.4800	3.4337	37.7418	6.2541	5.2117	1.4907
M-2-6	Meyers	2	3		1.2220	148.2653	1345.3173	2.9770	2.1054	49.2938	140.7292	2.3115	3.6562	-8.3072	6.0364	1.2813	0.9505
M-2-7	Meyers	2	4		1.8805	349.2547	2092.7290	3.7998	2.8244	59.5782	194.0934	3.6788	3.9777	6.9615	1.7278	3.9734	3.4485
M-2-8	Meyers	2	4		2.9735	513.0336	3556.7890	5.3417	6.3615	90.3368	190.1658	4.3629	5.3110	-13.7578	8.9258	4.3785	2.8676
M-3-1	Meyers	3	1		0.2660	23.2559	975.0227	3.0613	1.0625	79.7077	167.7420	4.1842	5.3411	7.7450	2.3036	4.5172	0.6910
M-3-2	Meyers	3	1		0.1988	50.7466	1173.0154	2.8648	1.1770	81.2941	165.5578	4.4506	4.8710	4.5282	2.3894	4.6647	0.6116
M-3-3	Meyers	3	1		0.1299	30.6077	988.9266	2.9149	1.0566	71.1976	161.5861	3.8286	5.3622	3.4065	2.0386	3.9974	0.4771
M-3-4	Meyers	3	2		0.2368	73.2704	1129.4614	3.2295	1.1593	60.0216	189.9445	3.5249	3.8869	-1.8682	2.5736	2.6082	0.4037
M-3-5	Meyers	3	2		0.1585	38.7969	962.3136	2.7993	0.7870	48.2053	179.7191	2.2836	3.2086	-4.0802	2.0200	3.7835	0.2620
M-3-6	Meyers	3	3		0.2522	76.6346	1091.8789	2.4193	1.0271	63.5479	148.6069	4.1388	5.1288	2.8591	2.4042	0.4235	0.7235
M-3-7	Meyers	3	3		0.4153	81.8761	1178.9173	2.6429	1.8185	67.6147	159.5363	4.4742	4.9019	4.9538	2.7357	5.8061	1.3463
M-3-8	Meyers	3	3		0.1031	34.2581	1099.4832	3.1174	1.1244	87.2843	172.2351	4.7520	5.3945	3.4892	2.7427	6.2747	0.5507
M-3-9	Meyers	3	4		0.2893	71.5280	1158.7193	2.8738	1.2429	82.4247	209.9484	4.6266	6.1968	-4.6616	2.4266	4.5929	0.4261
M-3-10	Meyers	3	4		0.2666	32.9512	1354.6200	2.8186	1.1158	72.8323	164.7224	4.0766	5.2189	-9.2365	7.4142	4.2261	0.6028
M-3-11	Meyers	3	4		0.1772	34.1740	551.2379	2.3360	1.2541	60.3994	147.2098	2.9091	3.8611	5.4246	1.8247	3.0215	0.6425

Appendix B.4. Tissue concentrations (µg/g dry mass) of resident *Corbicula fluminea* at Site A grouped by timepoint.

		Element Mass	V 51	Mn 55	Fe 57	Co 59	Ni 60	Cu 63	Zn 68	As 75	Se 77	Sr 88	Cd 114	Hg 200	Pb 208
ClamID	**Location**	**Timepoint**													
A-0-1	Site A	0	0.5742	20.3967	503.5628	3.8976	2.2641	99.3156	188.5137	4.0699	6.3523	15.0413	30.7098	0.1240	0.2591
A-0-2	Site A	0	0.9739	13.6421	275.7951	3.3573	7.2233	334.7941	196.5883	8.3622	24.2049	20.0175	35.9007	0.1292	0.8294
A-0-3	Site A	0	0.7792	10.6378	207.9472	3.5124	5.5324	312.1969	115.8078	8.1789	23.1854	6.7384	44.4447	0.1354	1.4258
A-0-4	Site A	0	1.4035	10.6701	397.7625	4.0838	5.7714	1606.8242	187.9340	6.8738	23.6623	29.6923	57.8363	0.4088	1.1154
A-0-5	Site A	0	0.5699	8.0258	159.6949	2.9047	5.6577	308.4187	148.3991	6.1517	16.9157	11.2631	35.1953	0.1081	0.5494
A-0-6	Site A	0	0.6089	8.3210	155.5266	2.3146	5.1490	416.5609	182.3028	7.2985	20.1408	13.5855	25.4604	0.1152	0.9702
A-0-7	Site A	0	0.6159	9.4418	179.2483	2.8094	5.8170	332.5984	116.0108	7.0320	19.5177	13.1330	37.7842	0.0939	0.5704
A-0-8	Site A	0	0.7357	10.0649	191.9846	3.6600	6.9611	318.8866	198.7848	8.0549	23.7288	16.5987	46.6572	0.1295	0.8791
AN-1-1	Site A	1	0.7174	34.3309	427.7456	5.5692	11.2064	428.5988	303.3197	11.5059	32.9255	17.3293	57.1940	0.1183	0.7938
AN-1-2	Site A	1	0.4121	20.8974	287.1738	2.9693	5.4856	381.3567	175.8216	6.6772	18.5518	5.5574	28.2137	0.0677	0.3548
AN-1-3	Site A	1	0.4411	21.0977	383.8644	3.6391	7.5100	215.0574	191.4410	7.5643	23.4975	3.6551	34.8725	0.0940	0.3576
AN-1-4	Site A	1	0.5466	28.4764	453.9268	4.3456	7.4794	850.3352	260.2766	8.5447	26.7292	4.4752	49.3003	0.0816	0.7177
AN-1-5	Site A	1	0.6865	35.1659	497.5402	5.4392	10.7259	247.3067	165.3011	7.7547	23.0972	4.4199	51.5913	0.0638	0.5888
AN-1-6	Site A	1	0.6295	29.2845	430.4159	3.6384	6.5327	533.9190	241.2695	8.1736	26.8608	7.2495	45.9508	0.0937	1.1028
AN-1-7	Site A	1	0.6472	62.6425	384.2629	4.9258	14.1139	140.6697	306.2482	6.7938	25.4844	2.9340	44.3718	0.0726	0.5032
AN-1-8	Site A	1	0.8315	46.6094	428.3725	4.6577	10.8815	174.2446	243.1785	8.6068	26.4876	2.6625	47.5677	0.0831	0.7263
AN-2-1	Site A	2	0.6576	10.6932	388.3317	2.0941	5.3282	145.9242	162.0958	9.5427	22.4358	11.6240	15.8347	0.0517	0.1616
AN-2-2	Site A	2	0.9030	9.9339	427.8211	2.3128	7.7123	280.9585	257.3073	8.6824	25.7907	20.9575	22.7639	0.0778	0.2441
AN-2-3	Site A	2	1.0426	16.0057	503.2712	3.2503	10.3477	211.4058	278.5951	9.9416	29.0609	30.3410	28.9416	0.1109	0.2775
AN-2-4	Site A	2	1.5671	14.5520	568.8237	2.6060	11.4398	96.8770	289.2609	8.2065	26.5352	46.2078	25.5211	0.0343	0.2902
AN-2-5	Site A	2	1.5564	17.8369	610.1679	2.8220	12.5372	138.6950	398.4201	7.7845	27.7781	37.7146	25.6916	0.0504	0.3479
AN-2-6	Site A	2	1.4689	13.4809	480.6851	2.9996	12.4244	119.0904	362.8812	7.6653	26.9955	32.7323	30.3910	0.0953	0.3620
AN-2-7	Site A	2	1.1472	16.7375	553.0438	2.7724	11.0792	94.6737	383.2506	9.4688	34.3232	47.2989	29.3527	0.0922	0.4734
AN-2-8	Site A	2	0.9610	7.0652	370.4127	2.1128	7.6173	480.8828	207.4863	9.5067	25.0254	16.2083	19.9209	0.1785	0.4370
AN-3-1	Site A	3	0.7493	7.8753	423.0904	1.6999	9.0881	52.1235	225.0355	8.6135	25.0441	59.5529	11.1259	0.0152	0.1825
AN-3-2	Site A	3	0.7579	6.6392	275.4112	1.7074	8.9109	75.2079	242.2763	9.1731	25.2448	19.6865	15.2054	0.0464	0.1807
AN-3-3	Site A	3	0.6298	9.0416	328.3148	2.1587	10.8715	109.9542	258.5286	9.1446	24.3108	34.0505	13.8161	0.0565	0.1791
AN-3-4	Site A	3	0.3898	5.3733	209.4198	1.3781	8.6322	257.5750	221.6037	9.8006	19.9417	11.3829	10.1736	0.1051	0.1335
AN-3-5	Site A	3	0.5898	9.0782	273.8681	2.0357	11.2437	101.4495	388.1339	9.3637	23.8878	26.5964	14.3712	0.0688	0.2243
AN-3-6	Site A	3	0.3650	4.7451	299.2087	2.3016	9.1480	163.8483	257.7716	10.0100	23.2521	19.9144	11.3036	0.1142	0.1347
AN-3-7	Site A	3	0.8619	10.7907	434.9186	2.5129	15.3957	63.3265	364.6805	9.2716	26.1121	57.4239	13.6812	0.1096	0.2848
AN-3-8	Site A	3	0.3661	6.3434	299.3562	3.1571	15.9085	165.7721	261.8288	10.2149	22.6603	16.9500	11.4437	0.1693	0.1616

Appendix B.5. Tissue concentrations (µg/g dry mass) of *Corbicula fluminea* collected in summer 2005 grouped by site and timepoint.

Site	ClamID	V 51	Mn 55	Fe 57	Co 59	Ni 62	Cu 63	Zn 67	As 75	Se 77	Sr 88	Cd 111	Hg 202	Pb 208
Site A1	A1C01	0.4982	21.8285	336.5078	2.4735	2.4707	56.6183	244.1490	5.9363	6.2614	10.9596	2.3325	0.1225	0.2810
Site A1	A1C02	0.3601	6.4738	225.2006	1.1422	1.0502	38.9045	140.6330	4.9722	5.6159	8.5224	1.7562	0.0804	0.1120
Site A1	A1C03	0.4367	15.2751	283.2966	2.3227	2.0507	63.6711	208.3931	5.8549	6.7837	9.5910	3.0672	0.1109	0.1690
Site A1	A1C04	0.4724	19.5096	344.5861	2.9642	2.3255	80.9344	246.6069	5.1679	6.4980	15.1075	3.4075	0.2107	0.1671
Site A1	A1C05	0.4751	20.4300	297.1125	2.0052	1.7045	56.1231	191.3786	5.2145	6.6413	10.6611	2.5816	0.0833	0.1677
Site A	AC01	2.8300	64.9081	568.0734	4.1798	7.8668	435.8256	225.9158	8.9357	19.5254	19.9388	21.5637	0.0690	1.5388
Site A	AC02	2.5699	68.3607	423.2747	7.5767	11.7150	619.7750	400.9356	10.8217	24.3319	30.0926	32.7853	0.1521	1.7384
Site A	AC03	1.8975	53.2582	468.5402	5.5280	8.6139	190.9052	284.5495	10.0485	21.5016	20.2486	31.6484	0.0266	1.0032
Site A	AC04	2.2074	51.2043	359.9909	3.6418	6.1766	251.1248	155.0742	8.4240	18.6892	18.5303	21.1214	0.0291	1.0963
Site A	AC05	1.9996	46.3683	336.9222	4.1690	7.6168	149.4072	168.6292	9.7763	19.8787	16.6343	23.2102	0.0257	0.9265
Site B	BC01	0.3485	11.9934	263.5411	2.0900	1.5967	28.5503	268.5571	3.3571	5.3867	11.2127	2.3254	0.0558	0.3543
Site B	BC02	0.4577	21.4290	488.7669	4.3120	4.5141	68.2175	407.0305	3.4925	7.2698	18.3494	6.4661	0.0717	0.7626
Site B	BC03	0.3356	13.0008	313.4952	1.3541	1.4011	48.6500	160.1931	3.7915	6.7992	11.5263	3.0602	0.0752	0.2472
Site B	BC04	0.5209	23.1427	396.0314	3.9158	3.9080	87.1917	422.9306	5.0301	9.0009	18.1433	6.5177	0.1734	0.5300
Site B	BC05	0.3806	27.8451	237.3835	2.1929	1.9059	52.0334	176.7043	5.0252	5.6614	9.0538	3.0095	0.0988	0.3395
Meyers	MC01	1.5136	378.9258	2159.3744	5.6792	2.7684	87.2235	157.3136	4.8153	4.4304	11.4640	3.3453	5.1101	1.8605
Meyers	MC02	1.2585	258.4526	2006.1929	5.8682	2.2794	86.6864	164.3024	4.5645	3.9991	12.0264	2.9729	5.8720	1.7433
Meyers	MC03	1.0348	174.3933	1548.1244	3.7953	1.5898	73.3914	158.3260	3.8727	4.0691	12.7509	2.9489	6.0253	1.5859
Meyers	MC04	1.3606	256.8732	1982.0563	4.9651	2.2878	68.6888	147.8515	4.0264	4.8068	13.4252	2.7297	5.3402	2.8700
Meyers	MC05	0.8385	178.6500	1170.2510	4.1511	1.7004	51.0479	102.6787	2.3316	2.3210	207.0634	1.2514	2.0923	1.0739

APPENDIX C

SUPPLEMENTAL MATERIAL TO CHAPTER 4

Appendix C.1. Trace element concentrations (µg/g dry mass) grouped by site and food web component.

Location	Sample ID	Biota Type	V 51	Mn 55	Fe 57	Co 59	Ni 62	Cu 63	Zn 67	As 75	Se 77	Sr 88	Cd 111	Hg 202	Pb 208
Meyers Branch	Meyer01	Water	0.4185	26.9746	382.3768	0.1887	0.1506	0.7470	38.0392	0.6096	0.2774	19.6305	0.0107	0.0457	0.1328
Meyers Branch	Meyer02	Water	0.3470	26.0183	338.1223	0.1433	0.1454	0.7141	6.5312	0.3436	0.2856	18.6632	-0.0085	0.0036	0.0642
Meyers Branch	Meyer03	Water	0.3857	26.1160	323.8514	0.1446	0.1890	0.3226	6.8745	0.3555	0.2305	18.8026	-0.0185	-0.0106	0.1982
Meyers Branch	CPOM-M1	Leaves	12.1785	7993.7800	13529.2957	34.3422	8.9114	8.0768	44.5900	5.1110	0.7870	40.6784	0.3175	0.0918	8.2116
Meyers Branch	CPOM-M2	Leaves	12.2871	11271.5880	16624.2218	38.7213	9.7424	11.3978	54.1794	8.0727	0.8978	49.3983	0.5068	0.1144	7.3049
Meyers Branch	CPOM-M3	Leaves	6.7063	7745.3937	11074.7564	25.0687	6.4778	9.1915	39.3818	3.9458	0.3786	45.5850	0.3034	0.0725	3.7953
100605A24	BOM-M1	Benthic Organic	22.72005	2355.40181	16748.1404	24.17725	9.583028	8.832043	54.82876	5.299487	0.780458	20.51992	0.401188	0.123878	18.87085
100605A25	BOM-M2	Benthic Organic	17.62098	1843.00844	11499.4874	17.75978	7.924381	6.828257	40.56644	4.144427	1.125749	18.69514	0.360541	0.21472	15.75823
100605A26	BOM-M3	Benthic Organic	24.00664	2979.67884	16701.8955	24.8863	11.73645	9.872313	55.29629	5.8532	0.979428	24.14564	0.455114	0.179204	19.95299
100605A2	BiofilmM1	Biofilm	7.980472	3819.00759	6731.73058	14.79954	3.874458	4.091731	26.8502	2.10254	0.523501	10.42798	0.988204	0.057129	15.61903
100605A4	BiofilmM2	Biofilm	7.129663	3008.83922	5179.43895	11.73455	3.036	2.953147	15.94153	1.651559	0.592117	7.838862	0.495165	0.040828	10.8725
100605A5	BiofilmM3	Biofilm	8.451617	4123.58157	7116.65403	15.69681	18.66491	50.35622	47.91651	2.04986	0.420422	10.39363	1.349921	0.040844	9.699569
Meyers Branch	SestonM1	Seston	24.1532	5707.8155	22594.0607	33.1515	11.6458	11.6526	59.5881	7.9429	0.9725	25.4629	0.4473	0.1644	20.4933
Meyers Branch	SestonM2	Seston	23.9063	5735.5145	22160.0672	32.9836	11.1286	9.6543	54.3769	7.8740	1.0670	26.0120	0.3969	0.1636	20.3018
Meyers Branch	SestonM3	Seston	22.4934	5524.8129	20971.6617	31.2808	10.6803	9.5160	52.9118	7.5294	0.9651	23.9994	0.3765	0.1483	19.1820
Meyers Branch	MC01	*Corbicula fluminea*	1.5136	378.9258	2159.3744	5.6792	2.7684	87.2235	157.3136	4.8153	4.4304	11.4640	3.3453	5.1101	1.8605
Meyers Branch	MC02	*Corbicula fluminea*	1.2585	258.4526	2006.1929	5.8682	2.2794	86.6864	164.3024	4.5645	3.9991	12.0264	2.9729	5.8720	1.7433
Meyers Branch	MC03	*Corbicula fluminea*	1.0348	174.3933	1548.1244	3.7953	1.5898	73.3914	158.3260	3.8727	4.0691	12.7509	2.9489	6.0253	1.5859
Meyers Branch	MC04	*Corbicula fluminea*	1.3606	256.8732	1982.0563	4.9651	2.2878	68.6888	147.8515	4.0264	4.8068	13.4252	2.7297	5.3402	2.8700
Meyers Branch	MC05	*Corbicula fluminea*	0.8385	178.6500	1170.2510	4.1511	1.7004	51.0479	102.6787	2.3316	2.3210	207.0634	1.2514	2.0923	1.0739
Meyers Branch	Hyda1	*Hydatophylax sp.*	1.1540	1478.1675	960.0759	6.5274	1.0996	13.0965	174.3385	0.6363	0.3247	5.7762	0.2219	0.0069	0.7994
Meyers Branch	Hyda2	*Hydatophylax sp.*	1.5326	1564.4184	1215.2453	6.4235	0.8798	11.9346	110.9201	0.8328	0.2379	8.7206	0.1800	0.0072	0.8267
Meyers Branch	Hyda3	*Hydatophylax sp.*	1.2781	945.4653	1146.1943	5.0317	1.0156	11.3190	162.0260	0.4212	0.2027	7.2523	0.1151	0.0250	0.8373
Meyers Branch	Hyda4	*Hydatophylax sp.*	1.8282	1639.8640	1397.7685	7.9210	1.7814	15.3432	212.4781	0.8574	0.1951	5.0346	0.2642	0.0039	0.7560
Meyers Branch	Hyda5	*Hydatophylax sp.*	1.6619	1160.5564	1436.6327	5.3182	1.2281	10.1186	154.9062	2.1009	0.4179	5.1376	0.3815	0.0782	1.5491
Meyers Branch	MG04	*Gomphus sp.*	1.7520	95.2432	1570.4501	1.8103	0.8416	15.0674	97.6131	1.5845	2.4743	2.3428	0.1789	0.2932	1.1618
Meyers Branch	MG05	*Gomphus sp.*	2.4690	254.1289	1105.9947	2.1745	0.7221	22.0683	91.0142	0.9589	1.8727	5.3444	0.2084	0.4177	2.3826
Meyers Branch	MG08	*Gomphus sp.*	1.0823	65.8372	499.3360	2.0407	1.2283	11.0101	104.1510	2.0232	3.4275	1.6792	0.2168	0.1846	4.6429
Meyers Branch	MG09	*Gomphus sp.*	3.0455	196.7877	3015.2600	3.0861	1.9928	32.5907	124.3566	1.9751	1.6333	4.0569	0.2221	0.5855	5.5319
Meyers Branch	MG10	*Gomphus sp.*	1.7056	109.8598	1441.6524	2.0396	1.1124	26.3080	134.9059	1.2143	2.3829	4.0993	0.1186	0.5418	3.4400
Meyers Branch	MCR01	Cambaridae	0.4647	508.6970	258.6047	1.9483	0.5612	26.2207	54.6126	0.5728	1.2279	260.5948	0.1376	0.1076	1.1321
Meyers Branch	MCR04	Cambaridae	0.4640	134.5760	242.6142	1.0183	0.2541	30.3097	50.4242	0.5506	1.8375	233.7732	0.0519	0.1782	0.7318
Meyers Branch	MCR08	Cambaridae	0.6909	658.6874	495.6092	2.8575	0.5155	31.5499	60.9076	0.6568	1.5575	295.3145	0.2288	0.0569	0.3105
Meyers Branch	MCR10	Cambaridae	0.6247	602.7456	346.5220	2.0662	0.6784	27.8271	56.4114	0.9571	1.2351	265.9538	0.2080	0.1027	0.4281
Meyers Branch	MCR12	Cambaridae	0.7010	856.6697	604.2242	3.0110	0.5015	21.6917	57.5198	0.7246	1.1761	267.3254	0.2555	0.0835	0.3544

Appendix C.1. Continued.

			V	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Sr**	**Cd**	**Hg**	**Pb**
Location	**Sample ID**	**Biota Type**	**51**	**55**	**57**	**59**	**62**	**63**	**67**	**75**	**77**	**88**	**111**	**202**	**208**
Meyers Branch	ME01	*Anguilla rostrata*	0.3754	35.9870	164.8474	0.4567	0.4311	12.0461	63.7801	0.3833	1.9477	22.6345	0.0829	0.7115	0.1788
Meyers Branch	ME02	*Anguilla rostrata*	0.3133	27.5300	249.1366	0.3776	0.9608	2.7550	58.8375	0.3652	2.3163	21.3861	0.0781	1.8257	0.1200
Meyers Branch	ME03	*Anguilla rostrata*	0.3030	33.1320	242.0044	0.3636	0.4860	2.0976	52.4096	0.5585	2.5959	20.2239	0.0448	0.5269	0.1368
Meyers Branch	ME04	*Anguilla rostrata*	-0.0514	43.6201	340.4136	0.4348	2.6214	3.4238	62.9489	0.4754	3.1049	30.9591	0.1102	1.2759	0.2785
Meyers Branch	ME05	*Anguilla rostrata*	0.0912	38.3494	293.4414	0.3450	1.1057	1.9905	51.4332	0.2725	1.9060	19.3176	0.0652	0.6584	0.1745
Meyers Branch	MC02	*Semotilus atromaculatus*	0.0620	16.9133	149.5551	0.2440	0.4869	1.7342	68.2183	0.0683	1.0159	24.0653	0.0413	0.2953	0.0392
Meyers Branch	MC04	*Semotilus atromaculatus*	0.2474	83.1668	212.3236	0.6893	0.6971	8.0891	90.8681	0.5662	2.1812	60.4284	0.1099	0.6177	0.0140
Meyers Branch	MC05	*Semotilus atromaculatus*	-0.6143	27.2288	457.6422	0.5476	2.2749	5.7959	138.2814	0.3600	1.9729	50.6592	0.0792	0.6098	0.1847
Meyers Branch	MC07	*Semotilus atromaculatus*	0.3603	72.4129	171.6352	0.4841	0.3598	5.8522	66.6780	0.3416	1.3008	45.9980	0.0599	0.4144	0.1651
Meyers Branch	MC08	*Semotilus atromaculatus*	0.5164	102.5016	224.1442	0.6907	0.6029	5.1332	109.5171	0.4347	1.9101	70.3992	0.0838	0.4450	0.0467
Meyers Branch	MY06	*Notropis lutipinnis*	-0.0120	53.8041	186.2857	0.3108	9.4920	4.1843	188.6542	0.1064	1.4832	51.7706	0.0278	0.3026	0.1242
Meyers Branch	MY07	*Notropis lutipinnis*	-0.3683	62.0140	312.7327	0.5630	21.8091	3.7045	237.4378	0.1582	1.8331	59.2651	0.0250	0.4207	0.1617
Meyers Branch	MY10	*Notropis lutipinnis*	-0.3396	56.2134	219.6540	0.4322	44.7102	7.3037	202.0476	0.4960	1.3362	43.4693	0.0445	0.3877	0.1251
Meyers Branch	MY19	*Notropis lutipinnis*	0.7765	57.3117	166.3806	0.3587	5.1336	2.7805	176.5167	0.0421	1.1496	49.8924	0.0706	0.3743	1.7304
Meyers Branch	MY15	*Notropis lutipinnis*	-0.1843	51.8749	407.7538	0.5412	38.4751	5.1493	221.6002	0.2037	1.5350	48.8920	0.0406	0.3637	0.1246
Meyers Branch	MR01	*Lepomis auritus*	0.4776	28.6934	154.2100	0.3856	0.6221	1.4349	81.5655	0.5245	2.6587	54.8097	0.0232	0.3522	0.1472
Meyers Branch	MR02	*Lepomis auritus*	0.7101	26.8897	112.2028	0.3591	0.1663	0.7829	55.6240	0.3570	3.5626	40.5735	0.0306	0.4624	0.0528
Beaver Dam Creek	SiteA01	Water	10.6203	44.2929	-0.9471	0.2913	4.8320	2.8547	12.0826	10.9189	3.2079	106.1067	0.2637	-0.0161	0.0163
Beaver Dam Creek	SiteA02	Water	11.1857	45.6913	11.0430	0.2956	5.0408	3.0571	15.3288	11.2870	2.9862	111.3848	0.2251	-0.0100	0.1620
Beaver Dam Creek	SiteA03	Water	12.1114	47.7889	2.0450	0.3223	5.5993	3.9883	42.3478	12.4359	3.7242	126.2706	0.2581	-0.0102	0.0181
Beaver Dam Creek	CPOM-A1	Leaves	43.1715	4040.5816	3473.9100	49.0081	118.2388	95.6739	167.4801	51.1040	7.7840	89.2318	10.2667	0.3575	7.2334
Beaver Dam Creek	CPOM-A2	Leaves	59.9686	4275.9972	3713.0003	50.2569	145.6151	95.5204	161.5944	47.3001	9.9901	85.5261	11.6977	0.4121	8.7997
Beaver Dam Creek	CPOM-A3	Leaves	12.8628	8428.4689	13855.4415	35.3183	9.2835	8.5853	47.7236	5.4209	0.6231	41.9114	0.3465	0.0938	8.8778
Beaver Dam Creek	BOM-A1	Benthic Organic	81.4438	672.9919	7944.6468	46.0839	91.6991	229.4762	183.0414	103.7138	10.2085	49.9147	9.8741	0.2822	18.7565
Beaver Dam Creek	BOM-A2	Benthic Organic	170.4142	729.9259	11344.9970	41.9960	90.0499	233.6081	194.5921	141.9888	11.8143	57.2043	9.9968	0.3545	17.5257
Beaver Dam Creek	BOM-A3	Benthic Organic	93.9240	781.9276	9254.1067	47.0834	92.1514	277.3501	201.3984	140.1467	9.1624	45.3535	9.2530	0.2953	22.4117
Beaver Dam Creek	BiofilmA1	Biofilm	76.7861	9311.6334	5875.3046	115.7268	150.4478	173.6844	238.7676	127.4751	7.8463	36.4706	13.4272	0.4698	14.3419
Beaver Dam Creek	BiofilmA2	Biofilm	79.6397	9232.1053	6833.9068	117.8539	178.6416	215.6758	283.3043	145.1489	7.8358	42.7582	16.1794	0.4720	16.5710
Beaver Dam Creek	BiofilmA3	Biofilm	85.1990	9624.9142	8296.2385	121.3120	162.1656	188.7242	248.6385	146.5105	6.4905	38.9618	14.3066	0.3913	19.4395
Beaver Dam Creek	SestonA1	Seston	36.0157	562.3687	5515.1648	23.9178	74.4932	169.1191	161.8540	57.0095	5.5611	44.9464	8.3101	0.2823	18.0416
Beaver Dam Creek	SestonA2	Seston	30.8203	746.4296	4290.2698	24.3475	61.3661	133.8555	132.3524	50.3288	4.2974	32.5663	16.6439	0.1591	13.6622
Beaver Dam Creek	SestonA3	Seston	39.6230	654.9971	6183.2694	26.3299	81.7941	188.1632	193.6448	67.0369	5.8955	44.8215	9.3976	0.2197	19.9782
Beaver Dam Creek	FilamA1	Filamentous algae	76.1028	785.4057	6515.6853	28.2260	84.0491	170.4919	208.5824	65.3598	10.8469	93.4905	10.2690	0.2445	18.8831
Beaver Dam Creek	FilamA2	Filamentous algae	22.6104	1489.8526	2073.6227	21.8643	44.5283	63.4647	134.0486	34.2475	7.1012	60.0801	4.2995	0.1137	5.1928

Appendix C.1. Continued.

Location	Sample ID	Biota Type	V 51	Mn 55	Fe 57	Co 59	Ni 62	Cu 63	Zn 67	As 75	Se 77	Sr 88	Cd 111	Hg 202	Pb 208
Beaver Dam Creek	AC01	*Corbicula fluminea*	2.8300	64.9081	568.0734	4.1798	7.8668	435.8256	225.9158	8.9357	19.5254	19.9388	21.5637	0.0690	1.5388
Beaver Dam Creek	AC02	*Corbicula fluminea*	2.5699	68.3607	423.2747	7.5767	11.7150	619.7750	400.9356	10.8217	24.3319	30.0926	32.7853	0.1521	1.7384
Beaver Dam Creek	AC03	*Corbicula fluminea*	1.8975	53.2582	468.5402	5.5280	8.6139	190.9052	284.5495	10.0485	21.5016	20.2486	31.6484	0.0266	1.0032
Beaver Dam Creek	AC04	*Corbicula fluminea*	2.2074	51.2043	359.9909	3.6418	6.1766	251.1248	155.0742	8.4240	18.6892	18.5303	21.1214	0.0291	1.0963
Beaver Dam Creek	AC05	*Corbicula fluminea*	1.9996	46.3683	336.9222	4.1690	7.6168	149.4072	168.6292	9.7763	19.8787	16.6343	23.2102	0.0257	0.9265
Beaver Dam Creek	Macro1	*Macronema sp.*	7.8708	396.9679	401.6364	5.3316	2.5373	40.2443	130.6038	6.5372	18.8956	12.2772	4.8563	0.1906	6.6938
Beaver Dam Creek	Macro2	*Macronema sp.*	8.0829	406.0244	475.0012	5.5633	4.2431	40.7368	120.5534	6.7047	16.0884	12.9943	4.2320	0.1574	3.6328
Beaver Dam Creek	Macro3	*Macronema sp.*	6.0826	308.6472	288.0535	5.2582	2.5507	40.3881	120.3828	5.5200	19.8546	9.6422	3.4104	0.1505	2.1031
Beaver Dam Creek	Macro4	*Macronema sp.*	4.8347	275.5597	277.3968	5.2161	3.6924	39.3997	96.9619	6.0217	16.9618	9.2390	2.6740	0.1393	1.4784
Beaver Dam Creek	Macro5	*Macronema sp.*	3.1033	182.8371	87.0542	3.7379	2.5818	36.7867	79.1814	4.5555	19.3138	13.1079	2.0950	0.1682	0.4498
Beaver Dam Creek	AG02	*Gomphus sp.*	5.4137	89.5601	559.6596	8.6691	6.2320	66.6671	102.7109	27.2152	19.0200	8.9748	0.9851	0.0213	1.3834
Beaver Dam Creek	AG03	*Gomphus sp.*	6.6536	81.9348	671.3391	4.4156	4.5248	36.7892	92.4663	57.8662	15.4721	9.4172	1.1836	-0.0123	1.9522
Beaver Dam Creek	AG04	*Gomphus sp.*	4.8421	60.7841	396.4015	2.5596	1.2635	34.0562	85.7208	8.7405	18.3813	6.5736	0.7461	-0.0090	1.1179
Beaver Dam Creek	AG05	*Gomphus sp.*	8.0227	173.1835	1191.4542	6.6653	4.3514	35.4384	121.1180	18.8670	19.8382	18.2381	1.2703	0.0197	2.4212
Beaver Dam Creek	AG06	*Gomphus sp.*	10.7526	189.7866	1124.1223	5.7750	5.3857	53.5419	131.8266	16.9838	15.0066	14.4815	1.8603	0.0483	2.6083
Beaver Dam Creek	ACR13	Cambaridae	1.0102	180.3092	144.2201	2.0144	2.5501	207.7063	87.3711	4.2612	11.9325	828.0831	4.1728	0.0427	0.4779
Beaver Dam Creek	ACR17	Cambaridae	0.8786	215.3557	137.7168	2.0128	1.7500	194.7853	68.8558	3.7568	14.0287	848.7302	2.8300	0.0128	3.5317
Beaver Dam Creek	ACR19	Cambaridae	1.4280	160.7769	168.9795	2.1429	1.8122	188.8763	70.0068	4.6133	9.8459	944.3930	4.3905	0.0128	0.4746
Beaver Dam Creek	ACR20	Cambaridae	1.2857	194.8650	150.3895	3.3076	2.5521	172.2205	80.0732	4.2640	12.2274	677.3876	3.1566	0.0065	0.1802
Beaver Dam Creek	ACR21	Cambaridae	1.7849	181.2314	206.8260	2.1299	2.6708	213.4080	75.0171	5.5898	19.2972	716.6702	3.7471	0.0197	0.2479
Beaver Dam Creek	AE02	*Anguilla rostrata*	0.8375	68.6835	261.2945	0.6315	2.1533	7.7209	69.2724	1.1984	16.5572	29.4183	0.5419	0.6451	0.1737
Beaver Dam Creek	AE05	*Anguilla rostrata*	0.9774	32.8362	183.0388	0.6269	1.8691	6.6749	66.4363	1.1631	21.8612	34.7284	1.0130	0.0300	0.1653
Beaver Dam Creek	AE07	*Anguilla rostrata*	0.9126	74.3210	231.7316	0.8833	2.6958	7.8521	103.4549	1.3241	16.1304	34.2489	0.5533	0.3689	0.3400
Beaver Dam Creek	AE10	*Anguilla rostrata*	0.2917	53.1483	305.1557	0.6113	2.3629	4.0003	61.9423	1.0836	19.8385	28.7738	0.9987	0.0709	0.1138
Beaver Dam Creek	AE12	*Anguilla rostrata*	-0.0208	42.8810	327.6337	0.5512	5.3812	5.2890	57.8869	1.7170	25.6048	41.2715	0.8319	0.0955	0.2079
Beaver Dam Creek	AR02	*Lepomis auritus*	2.5442	48.7238	181.1081	0.6960	1.1822	3.8489	99.6267	2.2778	22.7459	173.5059	0.7738	0.1968	-0.1321
Beaver Dam Creek	AR03	*Lepomis auritus*	1.2063	42.2082	109.1734	0.7496	1.1661	3.4746	130.5371	1.8706	23.8376	164.6464	0.6001	0.0872	-0.0056
Beaver Dam Creek	AR06	*Lepomis auritus*	1.7182	42.0706	235.6873	0.7524	1.6287	2.4905	85.2271	1.9526	19.6906	161.2874	0.6528	0.1519	-0.1077
Beaver Dam Creek	AR07	*Lepomis auritus*	1.8372	55.3109	273.0919	0.7808	1.2482	5.2098	93.8347	1.7478	23.6494	165.4734	0.6107	0.1368	-0.0137
Beaver Dam Creek	AR12	*Lepomis auritus*	2.8422	82.7539	92.2747	0.7129	0.6735	1.9341	87.3458	1.9463	22.5233	176.1602	0.7374	0.4392	-0.3316
Beaver Dam Creek	AR14	*Lepomis auritus*	1.8342	30.4120	161.6610	0.5150	0.9727	1.7526	74.6687	1.4064	14.4032	110.4920	0.3340	0.0938	-0.0097
Beaver Dam Creek	AG03	*Gambusia holbrooki*	1.1387	65.2000	103.9580	0.4118	3.8977	4.6874	200.3783	2.0153	18.4526	144.1852	0.4664	0.0647	0.0521
Beaver Dam Creek	AG04	*Gambusia holbrooki*	1.7278	73.2023	133.4817	0.6536	6.7690	11.2599	180.0034	2.2881	20.9024	165.1877	0.7190	0.0493	0.0985
Beaver Dam Creek	AG05	*Gambusia holbrooki*	1.6256	88.9968	149.4895	0.9122	7.4430	8.2067	194.4998	3.4199	25.0371	172.5624	0.8306	0.0320	0.0751
Beaver Dam Creek	AG07	*Gambusia holbrooki*	0.6833	61.8677	190.5056	0.4624	11.7472	7.7023	194.2827	1.7958	20.1295	151.2764	0.5841	0.0422	0.0601
Beaver Dam Creek	AG13	*Gambusia holbrooki*	1.3535	104.5195	221.8691	0.9466	27.2776	12.6998	198.1449	2.8342	22.4430	174.6238	0.8083	0.0480	0.0503

APPENDIX D

SUPPLEMENTAL MATERIAL TO CHAPTER 5

Appendix D.1. Trace element concentrations (µg/g dry mass) in bluegill liver and muscle tissue grouped by location and tissue type.

Site	ID	Tissue	V 51	Mn 55	Fe 57	Co 59	Ni 60	Cu 63	Zn 66	As 75	Se 77	Sr 88	Cd 111	Hg 200	Pb 208
Morgan Falls	101F	Fillet	0.1402	0.5934	8.1716	0.0292	0.0778	0.8958	87.0047	0.0731	2.7223	-1.7125	-0.0285	0.1131	0.0034
Morgan Falls	102F	Fillet	0.0193	0.4248	9.0808	0.0265	-0.0046	0.4906	54.7126	0.0711	2.3633	-3.5597	-0.0020	0.6770	-0.0194
Morgan Falls	103F	Fillet	0.1491	7.4106	11.8719	0.1666	0.0840	0.7985	30.4870	0.1565	2.7587	-1.3307	-0.0222	0.2136	-0.0171
Morgan Falls	104F	Fillet	0.0961	1.4011	14.6128	0.1021	0.0897	0.8223	40.6769	0.0844	2.2186	-0.9553	-0.0263	0.3865	1.1216
Morgan Falls	105F	Fillet	0.0719	0.8795	9.4000	0.0518	0.0011	0.5703	31.8470	0.1423	2.8212	-3.1301	0.0125	1.5539	-0.0338
Atlanta Road	201F	Fillet	0.1323	3.8894	16.1318	0.0943	0.1221	1.0757	40.6102	0.1559	1.8784	-1.3283	-0.0117	0.1179	-0.0209
Atlanta Road	202F	Fillet	0.0330	1.5184	15.8953	0.0669	0.1442	0.8313	39.7443	0.0779	1.7619	-4.1234	0.0028	0.3528	-0.0241
Atlanta Road	203F	Fillet	0.2600	1.0297	32.1830	0.1179	0.2248	2.4696	58.9538	0.2002	4.0133	-1.1257	0.0086	0.2610	-0.0082
Atlanta Road	205F	Fillet	0.0869	0.9442	32.5310	0.0448	0.1014	1.0133	36.9217	0.2026	3.3696	-0.7702	0.0032	0.4143	-0.0057
Atlanta Road	206F	Fillet	0.1307	1.8615	22.9683	0.0552	0.1130	1.0483	48.8173	0.1485	3.0114	-0.8740	0.0053	0.3061	-0.0073
Hwy 166	303F	Fillet	0.1652	1.2710	19.0237	0.0595	0.0630	0.7492	39.5966	0.1131	3.1612	-1.1710	0.0039	0.2281	-0.0077
Hwy 166	305F	Fillet	0.0451	0.7065	7.6244	0.0671	0.0848	0.8081	28.6759	0.1071	4.1219	-4.8478	0.0342	0.3282	-0.0306
Hwy 166	306F	Fillet	0.1168	0.6143	11.9622	0.0330	0.2025	0.7140	32.5488	0.1520	3.7276	-2.0849	-0.0028	0.1696	-0.0141
Hwy 166	307F	Fillet	0.0840	1.0854	21.4508	0.0895	0.0746	0.6440	35.5877	0.0813	2.8578	-2.7029	0.0027	0.2761	-0.0314
Hwy 166	308F	Fillet	0.1049	0.6254	9.3374	0.0339	0.0444	0.6439	42.3792	0.1368	3.7947	-1.5827	-0.0138	0.0694	-0.0170
Franklin	401F	Fillet	0.2467	5.4798	57.3139	0.0385	0.1227	0.5938	37.0063	0.2537	3.3628	0.7173	-0.0296	0.0635	0.0024
Franklin	402F	Fillet	0.1346	2.2144	29.0034	0.0467	0.1195	0.5066	32.3717	0.2964	5.0041	1.4091	0.0045	0.1724	0.0533
Franklin	403F	Fillet	0.0459	0.5576	11.9130	0.0321	0.1522	0.4501	37.8972	0.1924	4.6267	-2.2648	-0.0009	0.1954	0.0553
Franklin	405F	Fillet	0.1320	0.7115	12.2127	0.0192	0.0310	1.6872	28.5212	0.1366	3.6202	-1.3310	-0.0235	0.1904	-0.0096
Franklin	406F	Fillet	0.0089	-0.2919	6.1376	0.0765	-0.0190	1.0160	48.0418	0.1855	3.6001	-10.7866	-0.0402	0.2764	-0.1380
Morgan Falls	101L	Liver	1.2788	60.0117	539.8222	1.2701	0.1396	6.2987	93.8761	0.5810	7.8096	-3.1294	0.4773	0.3605	0.0044
Morgan Falls	102L	Liver	2.9563	71.1541	756.1239	2.7408	0.2259	7.2702	122.4031	0.4743	8.3482	-3.9110	1.9724	0.4663	-0.0058
Morgan Falls	103L	Liver	0.5285	190.7919	414.1329	1.2461	0.3097	19.5204	83.7155	0.7211	6.4607	3.4756	0.4395	0.4073	-0.0085
Morgan Falls	104L	Liver	0.5682	98.1748	419.3301	1.7036	0.3438	11.2823	122.7323	0.6232	8.2913	-2.1338	0.5482	0.2694	0.1572
Morgan Falls	105L	Liver	4.4573	34.9427	1502.8322	1.6389	0.5688	14.3134	109.6725	0.5464	9.2784	3.1499	2.7267	0.6211	0.1600
Atlanta Road	201L	Liver	2.1998	67.0405	1700.3710	1.0638	1.1388	2.7445	109.1285	0.3411	4.4206	-21.6274	0.0594	0.2113	0.4331
Atlanta Road	202L	Liver	0.5498	150.3594	323.5987	1.1754	0.2294	6.5838	107.4250	0.5562	5.2190	-7.2041	0.3041	0.2833	-0.0174
Hwy 166	305L	Liver	0.4223	12.9211	458.9078	0.8869	0.1352	7.0190	58.6098	0.2754	6.3611	-4.1737	0.6579	0.1952	0.0619
Hwy 166	307L	Liver	0.5955	49.2397	655.5051	1.8739	0.6429	9.4501	128.3747	0.8466	8.8588	-7.3999	0.7017	0.6696	0.4181
Hwy 166	308L	Liver	1.1129	273.7655	1154.2757	2.0051	0.1979	5.8629	125.1454	0.3350	7.8818	-3.4406	1.5070	0.5135	0.0381
Franklin	401L	Liver	0.3580	2.3568	634.1648	0.4655	1.5159	12.3148	143.1336	0.8143	4.3115	-40.2429	0.4504	1.4971	0.5516
Franklin	402L	Liver	1.9270	22.5314	553.0765	1.4224	0.2879	9.2419	124.6104	1.9852	7.6300	-9.2496	0.5215	0.2873	-0.0208
Franklin	403L	Liver	0.5572	16.7380	400.9658	1.2124	0.1318	5.7276	97.2165	0.9049	6.8708	-10.8989	0.4831	0.5068	-0.0573
Franklin	404L	Liver	1.5804	48.7321	579.6618	0.8414	0.4075	9.0536	91.1044	1.2929	8.6251	-3.2478	1.2949	0.2616	0.1161
Franklin	405L	Liver	5.5943	19.2578	840.9213	0.8949	0.2028	9.0458	97.1601	0.9525	8.2258	-5.5919	1.6856	0.2724	0.0750
Franklin	406L	Liver	0.8332	30.0550	469.2506	0.7076	0.4726	5.7084	168.8005	1.9900	8.4455	-15.5705	0.5856	0.3976	0.0683