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Assessment of metal contamination in the biota of four rivers experiencing varying degrees of human impact

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Abstract Urbanization, agriculture, and other land transformations can affect water quality, decrease species biodiversity, and increase metal and nutrient concentrations in aquatic systems. Metal pollution, in particular, is a reported consequence of elevated anthropogenic inputs, especially from urbanized areas. The objectives of this study were to quantify metal (Cu, Al, Cd, Ni, and Pb) concentrations in the waters and biota of four streams in South Georgia, USA, and relate metal concentrations to land use and abiotic and biotic stream processes. Additionally, macrophytes, invertebrates, and fish were identified to assess biodiversity at each site. Metal concentrations in the three trophic levels differed among sites and species, correlating to differences in land use surrounding the rivers. The highest metal concentrations (except Al) were found in the streams most impacted by urbanization and development. Al concentrations were highest in streams surrounded by land dominated by forested areas. Metal content in macrophytes reflected metal concentrations in the water and was at least three orders of magnitude higher than any other trophic level. Despite metal concentration differences, all four streams contained similar water quality and were healthy based on macroinvertebrate community structure. This study provides insight into the impact of urbanization and the fate and effects of metals in river ecosystems with varying degrees of anthropogenic impact.

Keywords Streams · Land use · Metals · Biota · Water quality

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Introduction

Human alterations to landscapes can substantially influence stream and river chemistry and the input of contaminants (Correll 1983; Peierls et al. 1991; Correll et al. 1992; Jordan et al. 1997a, b; Pyati et al. 2012; Gong et al., 2013). Water quality changes in streams and rivers may persist for decades, thus altering habitat and affecting biotic communities (Behnke 1990; Vinson and Hawkins, 1998). Metal pollution, in particular, is problematic in many aquatic environments, especially in densely populated areas (Klein 1979).

Metals may be suspended in the water column for various time periods, depending on a variety of abiotic and biotic factors. In the water column, metals can form inorganic complexes, reversibly bind to organic and particulate matter, and be passed through the food chain (Di Toro, et al. 2001). Eventually, metals partition in the sediment over time; however, metals may be remobilized into the interstitial water by both physical and chemical disturbances. Many metals (e.g., aluminum, iron) occur naturally in sediment, and, as sediment composition changes with local geography and environment, so do the concentrations of these elements in water bodies. Anthropogenic contributions of metals disrupt the natural cycles of these elements, which can result in the unidirectional movement of dissolved metals into aquatic systems (Eisler 1988a, 1988b, 1996; Klee and Graedel 2004).

Anthropogenic land-based sources of metal contamination include industrial discharges into streams or rivers, agricultural runoff, domestic stormwater runoff, and sewage treatment discharge (Pratt et al. 1981; Guzman and Jimenez 1992; Gonzalez et al. 1999; Echols et al. 2009). Naturally occurring trace metals such as copper and nickel are essential micronutrients required by all organisms; however, in excess, these metals, as well as nonessential metals, such as cadmium and lead, may accumulate in organisms and cause adverse biological effects (Bielmyer et al. 2005; Bielmyer et al. 2006; Bury et al. 2003; Rainbow and Dallinger 1993).

Most waterborne metals exert toxicity by binding to and inhibiting enzymes on the gills or gill-like structures of aquatic organisms (Bielmyer et al. 2006; Bury et al. 2003). This leads to a disruption in ion and water balance in the organism and possibly death, depending on the metal concentration and exposure time. Ingestion of metal-contaminated diets can also result in intestinal metal accumulation and potential toxicity to the consumer (Bielmyer et al. 2005; Bielmyer and Grosell 2011). Decreased respiration, decreased reproductive capacity, kidney failure, neurological effects, bone fragility, mutagenesis (genetic mutation), and other effects have been observed in aquatic biota after metal exposure. Several water quality parameters can modify the toxicity of metals including dissolved oxygen (DO), dissolved organic carbon concentration, sulfide and chloride concentrations, pH, water hardness, and alkalinity, as well as other variables (Campbell 1995). Metal toxicity may therefore vary in different water bodies, reflecting the changes in water chemistry (Campbell 1995) as well as the organisms that reside there.

Agriculture and urban land usage can impact the metal concentrations found in aquatic environments. Excess metals may accumulate in biota to varying degrees and potentially cause devastating effects in aquatic ecosystems, such as reduced species diversity and modified community composition (Mangun 1989; Grubaugh and Wallace 1995; Lenat and Crawford 1994; Roth et al. 1996; Roy et al. 2003; Dallinger et al. 1987; Cardwell et al. 2002; Vardanyan and Ingole 2006; Mortimer 1985). Therefore, the objectives of this study were to (1) quantify metal concentrations in the waters and biota of four streams with varying order and levels of anthropogenic disturbance and (2) relate metal concentrations at multiple trophic levels (water-abiotic, macrophytes, macroinvertebrates, small fish, large fish) to land use differences and governing abiotic and biotic processes.

Methods

Field sites

The population of Lowndes County, Georgia, is 114,552 spread throughout 496.07 mile^2 (U.S. Census Bureau: State and County QuickFacts, 2012). In this project, four streams in Lowndes County, each with varying levels of anthropogenic impact, were studied including One Mile Branch, OMB; Sugar Creek, SC; Withlacoochee River, WIT; and Little River, LR.

OMB and SC are both predominantly surrounded by urban landscapes and have experienced substantial human alterations. OMB is a first-order stream and is susceptible to metal inputs from impervious surfaces and other urban inputs. Over 50 % of the land use surrounding OMB is classified as medium density residential, followed by 22 % as light industrial, commercial, and institutional, 18 % as heavy industrial and roadways, and only 8 % as forest, open, and park (City of Valdosta, 2010). OMB is surrounded with high levels of vegetation and organic debris. This river runs through the main parking area of Valdosta State University, and the sampling site is located at 30° 50′ 35.9″ N, 83° 17′ 52.7″ W. A beaver dam was also noted 100 ft upstream from sampling site. While the dam was not a focus of our study, it did reduce flows and water levels for part of our study. OMB and some other streams confluence into

SC. SC is a second-order stream that is surrounded by impervious surfaces as well as urban landscape. Approximately 37 % of the total land use surrounding SC is classified as medium density residential, 24 % as light industrial, commercial, and institutional, 17 % as forest, open, and park, and 16 % as heavy industrial and roadways (City of Valdosta 2010). The SC sampling site is located near a restaurant and parking lot with coordinates of 30° 51′ 40.6″ N, 83° 19′ 05.2″ W. This stream runs through residential areas as well as shopping centers in downtown Valdosta, GA. It has a large amount of foliage surrounding the stream and many large stones positioned on the banks as a form of bank restoration.

LR, a third order stream, is surrounded mainly by forest (40 %) interspersed with cropland (36 %) and pasture (18 %), with only slight urban (6 %) influence, as determined by the United States Department of Agriculture. SC and LR both merge into WIT (a fourth-order stream) at separate locations. LR, sampled at 30° 51′ 08.5″ N, 83° 20′ 49.2″ W, and WIT, sampled at 30° 42′ 43.0″ N, 83° 27′ 20.6″ W, are both surrounded by dense vegetation and trees line the banks. WIT is predominantly surrounded by wetlands (36 %), agriculture (27 %), and upland forests (14 %) and only to a small degree by urban and built-up (13 %) and rangeland (6 %) (Southwest Florida Water Management District 2009). All four streams are considered black water and limestone base streams. LR and WIT both contain a large number of limestone boulders as part of the bedload in both streams. For the purpose of this study, OMB and SC were considered metal-impacted sites and LR and WIT were considered reference sites (Fig. 1).

Field sampling

During two large sampling periods (October 2013 and December 2013), fish were collected from all four stream sites using a dip net to capture small fish and a seine net to capture larger fish. Multiple habitats were sampled so that each stream habitat was represented. Fish were transferred to the Valdosta State University laboratory and then euthanized using tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, Redmond, WA). During the same sampling periods, macrophytes were collected by hand from OMB and SC only, because macrophyte presence was low in LR and WIT. Given the clumped and sometimes sparse distribution of the macrophyte communities in our systems, macrophyte collection did not follow a standardized technique. Collections gathered each species represented in the system forming a representative sample. Bryophytes were collected from boulders of limestone located throughout each stream basin. Given the karst topography, boulders greater than 20 cm are numerous and common in these systems providing some of the primary habitat. It should be noted that macrophytes are a minor component to each system with the exception of OMB. However, in the areas where they did exist, they were able to alter flows and cause sedimentation. Macrophytes were refrigerated until they were processed (see below).

During these larger seasonal samplings, the pH, DO, temperature, and chloride were measured using a YSI Professional Plus meter with various probes (YSI®, Yellow Springs, OH, USA), and 4–8 L of water was collected via subsurface grab in 1 L polypropylene cubitainers at three different sites in the downstream to upstream direction of each stream. Additionally, in the other months through March 2014, the pH, DO, temperature, and chloride were measured and 2–4 L of water was collected in polyethylene cubitainers at one site at each stream (because of heavy rainfall) for further lab analysis.

Macroinvertebrate samples were collected from the OMB, SC, WIT, and LR using insect kick nets and by hand over a 200-m reach in the fall of 2013. Macroinvertebrates were collected until 100 individuals were captured.

Laboratory analyses

All fish were identified using Hoyer and Canfield (1994), and macrophytes were identified using aquatic plant identification manuals (University of Florida Center for Aquatic and Invasive Plants). Biological diversity was quantified by calculating diversity indices using plant, invertebrate, and fish data (Beck's Index, Shannon-Weiner, Simpson's Index of Diversity). After euthanasia, small fish, ranging in size from ~1.5 to 7.0 cm total length, were immediately dried in an oven at 65 °C for 24 h, weighed, acidified with trace metal grade nitric acid (Fisher Scientific, Pittsburg, PA), and heated in a water bath to 60 °C until complete digestion. Large fish, ranging in size from ~9.0 to 15.0 cm total length, were immediately dissected and the following organs removed: gills, heart, intestine, liver, gallbladder, and spleen. Each organ of the larger fish as well as all

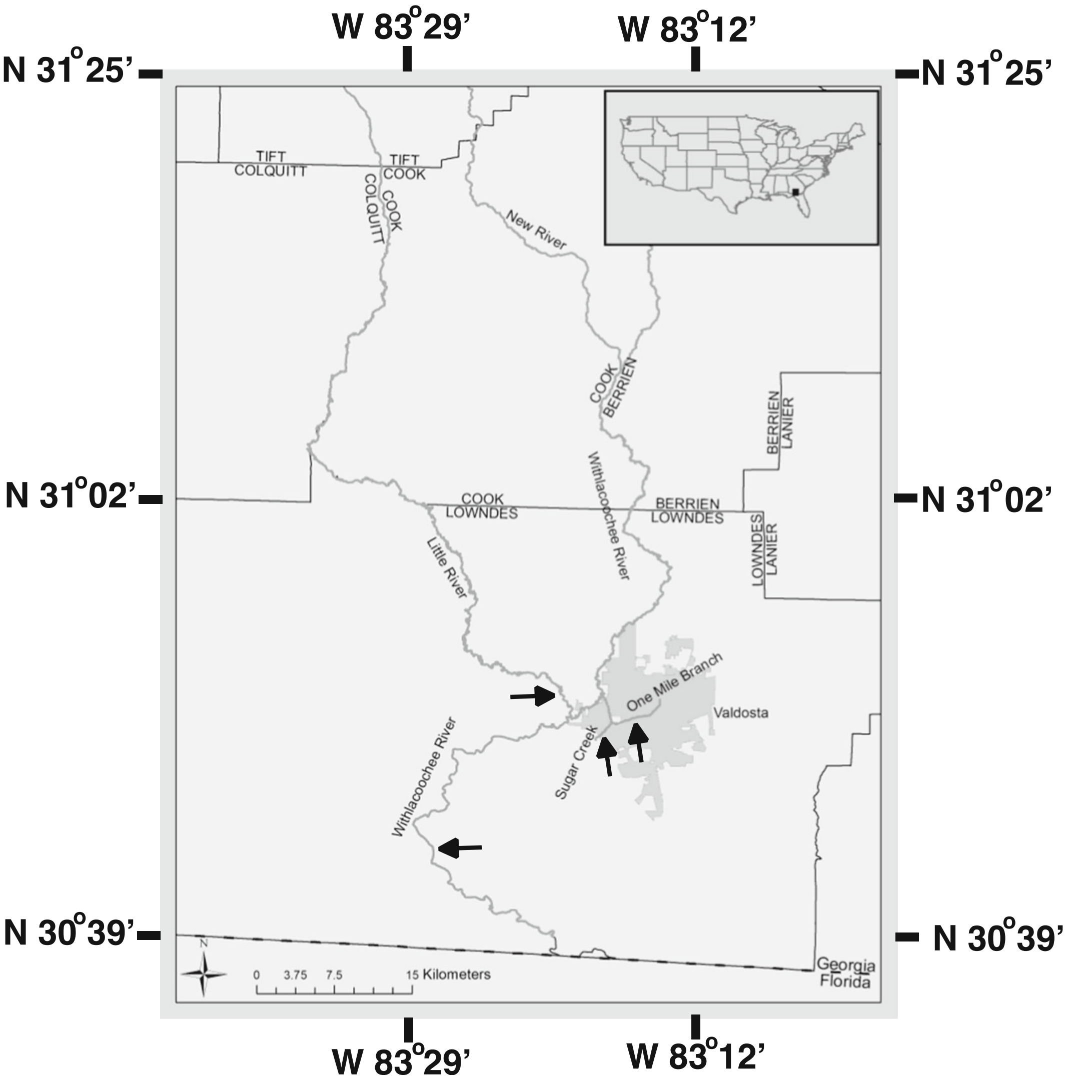

Fig. 1 Location of the sampling sites on four rivers in Lowndes County, GA

macrophyte (stem, leaves, roots) samples were dried and digested as described above. All digested tissue samples were diluted with 18 mΩ Milli-Q® water and then analyzed for Cu, Al, Cd, Ni, and Pb using atomic absorption spectrophotometry (AAS; PerkinElmer AAnalyst 800) with flame or graphite detection, as appropriate (detection limits ~1 ppb). These metals are frequently used in modern society and are thus commonly found in areas with increased anthropogenic disturbance. Certified 1 g/mL metal standards dissolved in 2 % HCl (Fisher Chemical, Fairlawn, NJ, USA) were used for each metal, and samples were analyzed in triplicate. The blank and standards were used for recalibration every 40 samples, and they were also analyzed as samples periodically throughout each cycle. Three replicates of a certified reference material (LUTS-1, lobster hepatopancreas) were treated as the samples to determine average extraction efficiencies. This method of digestion and metal analysis has been used in several other studies in our laboratory and has been effective (Shyn et al. 2012; Jarvis et al. 2013).

Water collected from each sampling site was filtered through pre-burned Whatman Glass Fiber Filters (0.7 μm) within an hour of returning to the laboratory using a vacuum-suction technique, and the filtrate was stored in polypropylene plastic bottles and refrigerated at 3 °C. Filtered water was measured for alkalinity, hardness, nitrite, phosphate, and carbon dioxide via a

LaMotte® Freshwater Colorimetric kit. Ammonia and nitrate levels were measured using a Red Sea Marine and Freshwater Test Kit®. Two subsamples (15 mL) of the filtered water from each site at each stream were additionally filtered (0.45 μm Nylon, Fisher Scientific, Pittsburg, PA), acidified, and then analyzed for Cu, Al, Cd, Ni, and Pb using AAS with graphite furnace detection.

Bioconcentration factors (BCFs) were calculated for each metal for each species of macrophyte at the two collection sites (OMB and SC). BCFs are expressed as the ratio of milligrams of metal in the macrophyte to the milligrams of metal per liter of water (environmental metal concentration; Hemond 2000). Higher BCF values are indicative of lower metal exposure levels (McGeer et al. 2003).

Macroinvertebrate samples were taken to the laboratory at Valdosta State University and immediately preserved in ethyl alcohol solution. All invertebrates collected were identified down to genus or to species if possible. Invertebrate data was used to determine stream health using the Beck's Biotic Index for stream health (Terrell and Perfetti 1996).

Statistical analysis

The waterborne metal data were analyzed for normality and equality of variance using a Shapiro-Wilk's test and a Bartlett's test, respectively. Statistical differences between sites and over time were calculated using an ANOVA followed by a post hoc Tukey's test with Sigma-Plot 11.0. Differences over time (October and December) were not observed; therefore, the data for the metal concentrations in each the macrophytes, smaller fish, and larger fish were combined for the two larger sampling periods.

Results

Water quality parameters are presented in Table 1. The pH (ranging from 5.72 to 7.05), alkalinity (ranging from 18 to 38 mg $CaCO_3$/L), and hardness (ranging from 22 to 58 mg $CaCO_3$/L) values were relatively low, and CO_2 (ranging from 3.9 to 11 mg/L) and DO (ranging from 5.1 to 11.7 mg/L) values were relatively high for freshwaters (Table 1). No substantial differences in these parameters were observed between sites; however, temporal changes were evident and correlated with an increase in rainfall (Fig. 2). Temperature varied by season with the coldest values observed in January. Alkalinity, CO_2, and DO values were highest in the winter months, which also related to an increase in rainfall (Table 1 and Fig. 2). The lowest pH values were measured in the spring when a peak in rainfall was observed (Table 1 and Fig. 2). Valdosta had an unseasonably wet period in December and January in particular (Fig. 2). Among the four streams, DO values were lowest in OMB, with the exception of April. Nitrogen concentrations, as ammonia, nitrate, and nitrite, as well as chloride concentrations were generally low at all sites (Table 1).

Metals were detected in every stream sampled; waterborne Al concentrations were the highest, followed by Cu, Pb, Ni, and Cd (Fig. 3). Significant differences in waterborne metal concentrations were observed among streams, with the highest Cu concentrations in the two metal-impacted streams and Al concentrations highest in the two reference streams (Fig. 3). In general, concentrations of all metals except Al were most elevated in OMB, whereas Al was highest in WIT and SC. A spike in Cu, Ni, Cd, and Pb concentration was observed in WIT in November (Fig. 3).

Macrophyte metal concentrations (Table 2) largely reflected the metal concentrations in the water and were up to several orders of magnitude higher than any other trophic level for some metals. Macrophytes were only found at two of the sites, OMB and SC, with far fewer species observed at SC as compared to OMB (Table 2). Significant differences in metal accumulation in macrophytes between sites were not determined because only one species (*Micranthemum* sp.) was found at both sites, and the sample sizes (n = 2–4) were too small of each species.

For the two sites that macrophytes were found, Al concentrations in the macrophytes were an order of magnitude higher than any other metal measured. Cu, Ni, and Pb accumulated to a similar extent, and Cd accumulated to the lowest degree in the macrophytes (Table 2). Bryophytes accumulated the highest metal concentrations in SC, whereas *Potamogeton* sp., *Eleocharis* sp., and *Micranthemum* sp. generally accumulated the highest metal concentrations in OMB (Table 2). Bioconcentration factors were calculated for each metal and are presented in Table 3. Considerable variation was observed among both macrophytes and metals. Additionally, for the species (*Micranthemum* sp.) that was found at both sites, BCF values

Table 1 Measured water quality parameters in four streams: One Mile Branch (OMB), Sugar Creek (SC), Little River (LR), and Withlacoochi River (WIT) over time

Water quality parameter	Sampling date	Site			
Dissolved oxygen (mg/L)		OMB	SC	LR	WIT
	October 2013	5.14 ± 0.69	7.07 ± 0.27	7.00 ± 0.03	6.29 ± 0.73
	November 2013	7.18 ± 0.14	8.85 ± 0.35	8.08 ± 0.59	8.12 ± 0.18
	December 2013	7.43 ± 0.14	9.68 ± 0.28	9.45 ± 1.35	8.56 ± 1.44
	January 2014	9.10 ± 0.00	11.7 ± 0.00	10.7 ± 0.00	9.70 ± 0.00
	February 2014	8.70 ± 0.42	9.65 ± 0.35	9.05 ± 0.35	9.20 ± 0.42
	March 2014	8.50 ± 0.00	9.60 ± 0.00	8.60 ± 0.00	8.10 ± 0.00
	April 2014	8.80 ± 0.00	8.10 ± 0.00	7.00 ± 0.00	5.70 ± 0.00
Temperature (°C)	October 2013	22.6 ± 0.4	23.5 ± 0.8	25.7 ± 0.0	23.8 ± 0.9
	November 2013	19.4 ± 4.3	17.6 ± 5.1	17.2 ± 3.7	18.1 ± 3.2
	December 2013	19.2 ± 0.4	17.3 ± 0.4	15.2 ± 2.2	15.4 ± 2.8
	January 2014	15.2 ± 0.0	11.3 ± 0.0	11.5 ± 0.0	12.0 ± 0.0
	February 2014	21.2 ± 1.1	20.2 ± 1.9	12.6 ± 0.6	12.9 ± 0.5
	March 2014	17.0 ± 0.0	14.3 ± 0.0	13.6 ± 0.0	14.3 ± 0.0
	April 2014	19.8 ± 0.0	18.0 ± 0.0	16.7 ± 0.0	17.0 ± 0.0
Carbon dioxide (mg/L)	October 2013	5.9 ± 2.9	3.9 ± 0.4	4.2 ± 0.4	5.3 ± 1.8
	November 2013	8.5 ± 4.4	4.0 ± 0.6	4.5 ± 1.0	6.0 ± 1.7
	December /2013	9.2 ± 3.4	4.3 ± 0.8	4.8 ± 0.7	5.7 ± 0.5
	January 2014	11 ± 0.7	6.0 ± 0.7	6.0 ± 0.7	7.0 ± 0.7
	February 2014	5.8 ± 1.0	4.3 ± 0.5	5.0 ± 1.2	5.0 ± 1.4
	March 2014	5.5 ± 1.0	5.5 ± 0.6	4.8 ± 0.0	5.0 ± 2.5
	April 2014	6.0 ± 0.0	5.0 ± 2.1	5.0 ± 0.8	5.0 ± 1.4
pH	October /2013	7.03 ± 0.12	6.96 ± 0.16	7.01 ± 0.02	6.89 ± 0.03
	November 2013	7.05 ± 0.04	6.97 ± 0.06	6.89 ± 0.09	6.95 ± 0.14
	December 2013	6.55 ± 0.61	6.80 ± 0.25	6.76 ± 0.32	6.73 ± 0.37
	January 2014	6.58 ± 0.00	6.91 ± 0.00	6.44 ± 0.00	6.56 ± 0.00
	February 2014	6.03 ± 0.08	6.41 ± 0.08	5.86 ± 0.04	5.95 ± 0.31
	March 2014	6.45 ± 0.00	6.01 ± 0.00	6.22 ± 0.00	6.23 ± 0.00
	April 2014	5.87 ± 0.00	5.99 ± 0.00	5.72 ± 0.00	5.77 ± 0.00
Alkalinity (mg $CaCO_3$/L)	October 2013	29 ± 7.7	38 ± 2.7	25 ± 1.2	30 ± 3.9
	November 2013	34 ± 3.0	38 ± 4.4	28 ± 3.3	38 ± 7.7
	December 2013	31 ± 1.7	35 ± 1.6	28 ± 2.7	31 ± 1.6
	January 2014	34 ± 0.0	36 ± 0.0	33 ± 0.0	32 ± 0.0
	February 2014	30 ± 2.3	34 ± 1.9	23 ± 6.0	19 ± 2.0
	March 2014	28 ± 0.0	30 ± 2.3	22 ± 2.3	20 ± 3.3
	April 2014	28 ± 0.0	32 ± 0.0	18 ± 2.8	22 ± 2.8
Hardness (mg $CaCO_3$/L)	October 2013	32 ± 21	58 ± 5.3	33 ± 1.7	38 ± 3.9
	November 2013	50 ± 5.2	53 ± 3.8	33 ± 3.9	48 ± 6.5
	December 2013	43 ± 2.2	44 ± 1.5	31 ± 1.5	36 ± 3.6
	January 2014	44 ± 0.0	42 ± 0.0	30 ± 0.0	26 ± 0.0
	February 2014	40 ± 3.3	44 ± 0.0	23 ± 2.0	22 ± 2.3
	March 2014	44 ± 4.6	44 ± 4.6	26 ± 5.2	24 ± 3.3
	April 2014	42 ± 2.28	42 ± 2.83	22 ± 2.83	22 ± 8.48
Chloride (mg/L)	October 2013	1.60 ± 0.22	1.60 ± 0.33	1.60 ± 0.00	2.10 ± 0.17

Table 1 (continued)

Water quality parameter	Sampling date	Site			
	November 2013	1.13 ± 0.18	1.19 ± 0.11	1.24 ± 0.16	1.48 ± 0.17
	December 2013	2.41 ± 0.29	2.38 ± 0.06	1.82 ± 1.10	1.28 ± 1.09
	January 2014	1.07 ± 0.00	1.22 ± 0.00	1.98 ± 0.00	1.58 ± 0.00
	February 2014	1.03 ± 0.19	1.09 ± 0.36	1.78 ± 0.05	1.29 ± 0.21
	March 2014	1.48 ± 0.00	1.31 ± 0.00	1.02 ± 0.00	0.94 ± 0.00
	April 2014	0.46 ± 0.00	0.38 ± 0.00	0.37 ± 0.00	0.45 ± 0.00
Ammonia (mg/L)	October 2013	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.8 ± 0.3
	November 2013	0.8 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.1
	December 2013	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
	January 2014	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.4 ± 0.1
	February 2014	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.4 ± 0.1
	March 2014	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
	April 2014	0.2 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.7 ± 0.0
Nitrite (mg/L)	October 2013	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
	November 2013	0.03 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
	December 2013	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
	January 2014	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
	February 2014	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
	March 2014	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
	April 2014	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.00
Nitrate (mg/L)	October 2013	1.50 ± 0.15	1.14 ± 0.17	1.22 ± 0.05	2.29 ± 0.31
	November 2013	1.63 ± 0.00	1.40 ± 0.00	0.93 ± 0.01	2.36 ± 0.18
	December 2013	1.63 ± 0.26	1.27 ± 0.15	1.60 ± 0.31	2.03 ± 0.23
	January 2014	1.69 ± 0.00	1.83 ± 0.00	2.02 ± 0.00	2.32 ± 0.00
	February 2014	1.63 ± 0.00	1.84 ± 0.00	1.98 ± 0.00	2.32 ± 0.00
	March 2014	1.92 ± 0.00	2.35 ± 0.00	2.53 ± 0.00	2.62 ± 0.00
	April 2014	1.71 ± 0.00	1.54 ± 0.00	2.42 ± 0.00	2.44 ± 0.00

substantially differed, and differences were not consistent among metals (Table 3).

Metals were also observed in the tissues of higher trophic levels. Metal concentrations in the smaller fish were most elevated in OMB, with the exception of Pb in Little River (Fig. 4). Of all the metals, tissue Al concentrations were the highest in the whole body of smaller fish, similar to that observed in the water and macrophytes (Fig. 4). Of the other metals, Cu was also particularly elevated in the whole body of smaller fish. *Gambusia holbrooki* followed by *Heterandria formosa* generally had the highest metal body burdens and were found in all four streams (Fig. 4).

Metals were also detected in the organs of the larger fish and varied depending on the metal and fish species (Fig. 5). Tissue metal concentrations were highest in the heart and liver of the spotted sunfish, the liver of the warmouth, the liver and gallbladder of the black crappie, and the heart and liver of the redbreast sunfish (Fig. 5). Tissue metal concentrations varied depending on the metal, in the redear sunfish (Fig. 5). For each metal, tissue metal distribution differed with fish species (Fig. 5). The redbreast sunfish, which was found in LR, had the highest tissue metal concentrations (with the exception of Al). The redear sunfish, found in SC, had the highest tissue Al concentrations and had comparable tissue Ni concentrations as those observed in the redbreast sunfish (Fig. 5).

No significant changes in species biodiversity were observed among the four sites using Shannon-Weiner's Index or Simpson's Index of Diversity (Table 3). All four streams were represented as stream clean or stream clean

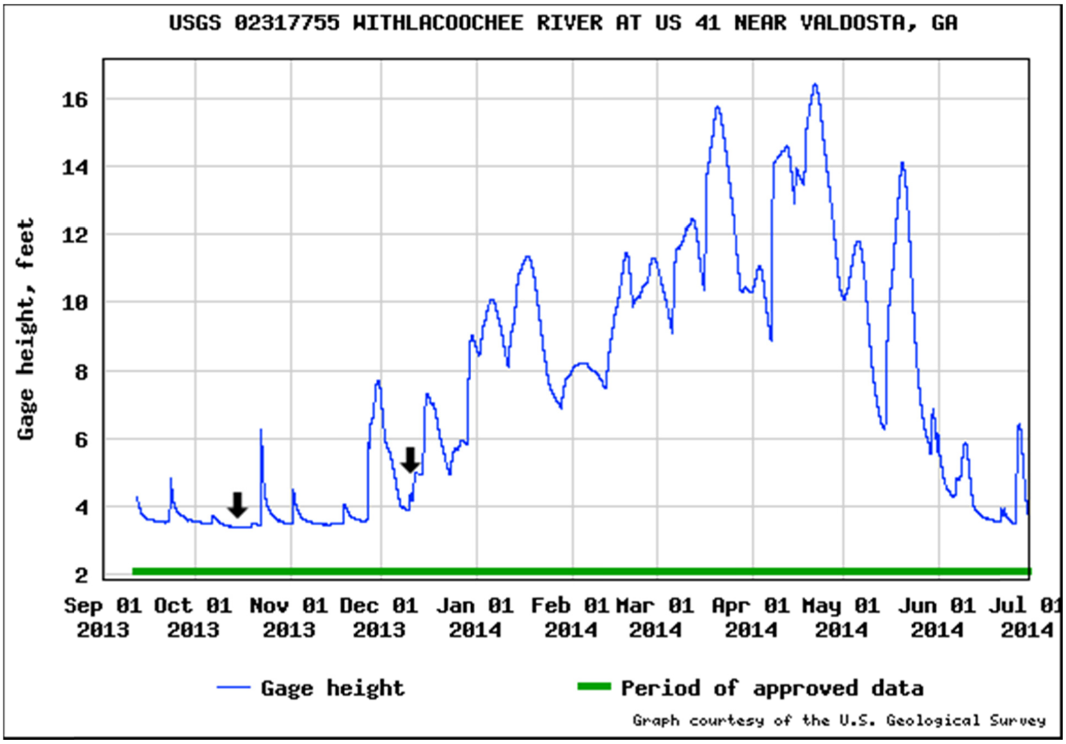

Fig. 2 Gage height of the Withlacoochee River during the sampling period. *Arrows* indicate intense sampling when biota was collected

in a monotypic habitat according to Beck's Index Score (Table 4). Macroinvertebrate samples from SC and LR were all group one taxa for the Beck's Index indicating good water quality (Table 5). The majority of macroinvertebrate samples collected from OMB and WIT were group 1 taxa with a few macroinvertebrate samples representing group 2 taxa indicating fair water quality.

Discussion

Metal concentrations found in the four streams sampled are comparable to those reported in other blackwater rivers (Pyati et al. 2012). In addition, a survey study in the nearby Alapahoochee River found low metal (Cu, Zn, Pb) concentrations near detection limits with the exception of sampling times following rain events (Barnett et al. 2007). However, the Alapahoochee River does not contain a direct urban influence in contrast to our study sites. OMB and SC are most impacted by urbanization and development so it follows that the metals associated with anthropogenic sources would be most elevated in these streams. OMB is also more shallow and was likely influenced by rainfall more than the other streams. The spike in Cu, Ni, Cd, and Pb concentration observed in WIT in November was likely due to excessive rainfall and thus increased inputs of nonpoint source pollution, a trend supported by other studies in the area (Barnett et al. 2007). The land use surrounding WIT and LR is dominated by forested and wetland areas mixed with agriculture (Southwest Florida Water Management District 2009). Al is a major component of clay, which is abundant in those areas. High amounts of Al have been shown to be naturally deposited in the sediments of nearby Long Pond, Lake Park, GA prior to human impacts throughout the past 4000 years (Earley 2015). Acidic rain can liberate Al from soils, and atmospheric deposition is believed to be the main cause for the acidification of freshwaters (Schuurkes et al. 1986; Van Breemen and Van Dijk 1988). Al depletion in forests can cause radical changes in soil genesis and may also lead to future reduction in acid neutralization (Mulder et al. 1989). The water chemistry observed in the streams also changed in response to the increased rainfall. The low alkalinity, hardness, and pH values in these streams could have facilitated increased bioavailability and uptake of the metals into the biota.

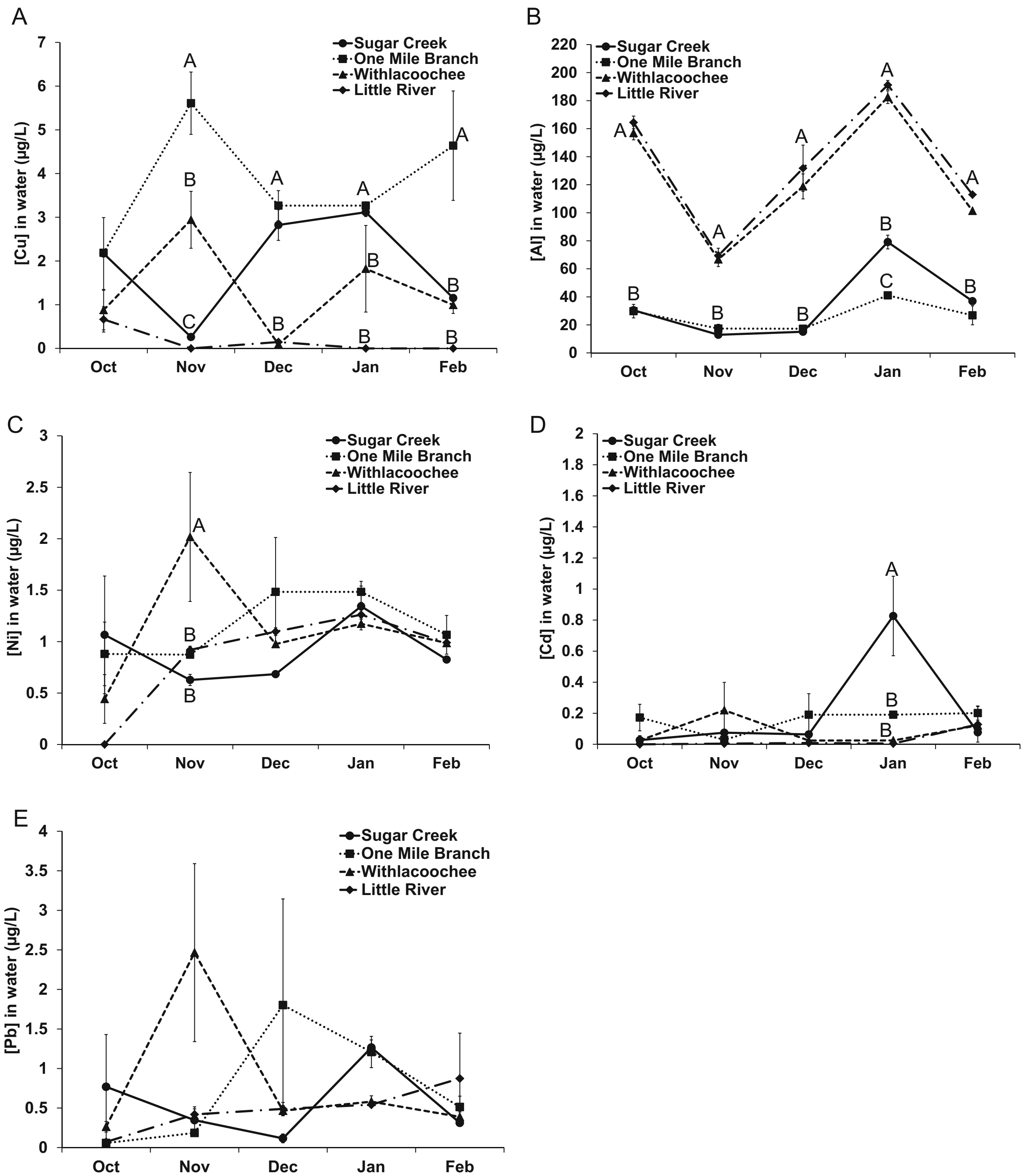

Fig. 3 Concentration (mean ± standard error; $n = 6$ in October and December; $n = 4$ in November, January, and February) of **a** copper (Cu), **b** aluminum (Al), **c** nickel (Ni), **d** cadmium (Cd), and **e** lead (Pb) in water samples from four streams: Sugar Creek, One Mile Branch, Little River, and Withlacoochee in 2013–2014. *Different letters* indicate significant differences in metal concentration between the streams in a given month ($p \leq 0.05$)

Macrophytes, invertebrates, and fish have been shown to accumulate metals to varying degrees and be susceptible to metal toxicity (Dallinger et al. 1987; Cardwell et al. 2002; Vardanyan and Ingole 2006;

Table 2 Concentration (μg/g dw) of copper (Cu), aluminum (Al), nickel (Ni), cadmium (Cd), and lead (Pb) in macrophytes collected from three sites along two rivers, One Mile Branch (OMB), and Sugar Creek (SC) in October 2013

Macrophyte ID	OMB [Cu]	SC [Cu]	OMB [Al]	SC [Al]	OMB [Ni]	SC [Ni]	OMB [Cd]	SC [Cd]	OMB [Pb]	SC [Pb]
Potamogeton sp.	201		8803		63		18		129	
Bryophyte		798		11,529		1214		95		330
Utricularia sp.	83.1		1542		16		3		62	
Micranthemum sp.	323	134	7826	3860	18	39	5	17	165	99
Eleocharis sp.	744		7074		108		15		283	
Nymphaea odorata	53.1		225		21		9		7	
Myriophyllum sp.	372		5267		150		12		131	

Mortimer 1985). In this study, macrophytes were only found at the two metal-impacted sites (OMB and SC), but there presence was not assumed to be a response to metal inputs. The magnitude of metal accumulation in the macrophytes was largely species dependent and similar to other reported studies (Förstner and Wittmann 1983; Cardwell et al. 2002; Kumar et al. 2008; Sanches Filho et al. 2015). Mortimer (1985) reported a higher bioconcentration of Pb than Ni or Cu in freshwater macrophytes. Venkatesha et al. (2013) reported accumulation of metals in the order of Cu > Pb > Ni > Cd. *Potamogeton* spp. have been shown to accumulate 242–380 μg/g dw Zn, 45–50 μg/g dw Cu, 5.8–7.5 μg/g dw Pb, and 1.2–2.2 μg/g dw Cd (Förstner and Wittmann 1983). Similar to that observed in the present study, Cardwell et al. (2002) reported a Cd concentration in *Myriophyllum aquaticum* of 6.5 μg/g dw.

Macrophytes have been used as biomonitors of metal pollution in freshwater environments for quite some time (Mortimer 1985; Phillips and Rainbow 1993). Additionally macrophytes are known to be more tolerant to metals than are aquatic animals (Tyler et al. 1989). Bryophytes contained some of the highest tissue metal concentrations. They have been reported in other studies to be a particularly useful bioindicator of metal pollution (Whitehead and Brooks 1969; Phillips and Rainbow 1993). The BCFs for macrophytes in this study (Table 2) were several times greater than those reported by Ndeda and Manohar (2014). However, other researchers have reported much higher (several orders of magnitude greater) BCF values (McGeer et al. 2003; Donnachie et al. 2014; Jarvis and Bielmyer-Fraser 2015), similar to those in the present study (Table 2). Even though macrophytes are generally more tolerant of metals, toxic effects have been reported. Elevated Cu has been shown to reduce photosynthesis and respiration rates in aquatic macrophytes (Vazquez et al. 2000). Jarvis and Bielmyer-Fraser (2015) reported phytotoxicity in *Ulva lactuca* after exposure to 100 μg/L of Cu or Cd or after exposure to a 100-μg/L mixture of Cd, Cu, Pb, Ni, and Zn.

Table 3 Bioconcentration factors (BCF) for accumulation of metals in the macrophytes collected from two streams, One Mile Branch (OMB) and Sugar Creek (SC)

Macrophyte ID	Cd OMB BCF	Cd SC BCF	Pb OMB BCF	Pb SC BCF	Cu OMB BCF	Cu SC BCF	Ni OMB BCF	Ni SC BCF	Al OMB BCF	Al SC BCF
Potamogeton sp.	103,000		2,230,000		92,000		71,000		295,000	
Bryophyte		3,530,000		430,060		368,000		1,135,000		379,000
Utricularia sp.	18,300		1,070,000		38,000		19,000		52,000	
Micranthemum sp.	27,900	623,000	2,840,000	129,239	148,000	62,000	20,000	37,000	263,000	127,000
Eleocharis sp.	88,700		4,875,385		340,000		123,000		237,000	
Nymphaea odorata	50,500		117,816		24,000		24,000		7550	
Myriophyllum sp.	67,400		2,251,499		170,000		170,000		177,000	

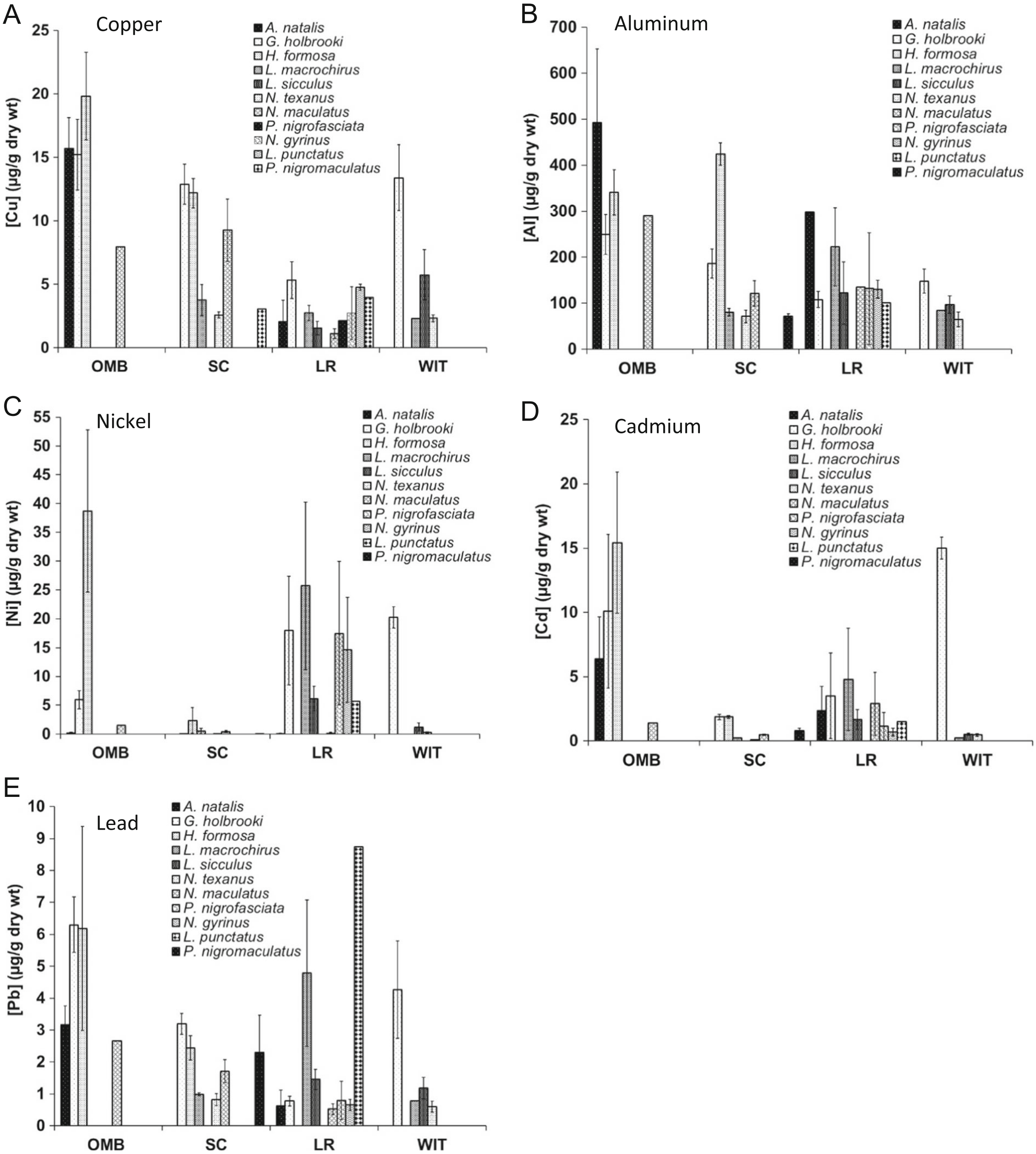

Fig. 4 Concentration (mean ± standard error) of **a** copper (Cu), **b** aluminum (Al), **c** nickel (Ni), **d** cadmium (Cd), and **e** lead (Pb) in small fish (1.5–7.0 cm) collected from four streams: Sugar Creek (*SC*), One Mile Branch (*OMB*), Little River (*LR*), and Withlacoochee (*WIT*). The fish collected include *Gambusia holbrooki* (eastern mosquitofish), *Labidesthes sicculus* (brook silverside), *Percina nigrofasciata* (blackbanded darter), *Noturus gyrinus* (tadpole madtom), *Lepomis macrochirus* (bluegill), *Lepomis punctatus* (spotted sunfish), *Heterandria formosa* (least killifish), *Notropis maculatus* (taillight shiner), and *Pomoxis nigromaculatus* (black crappie)

Tissue metal concentrations in small fish were generally highest in the most urbanized (metal-impacted) site (OMB). The Cu and Ni concentrations in the small fish were similar to those reported in laboratory toxicity studies (Bielmyer et al. 2005; Bielmyer et al., unpublished). Also, the small fish species *H. formosa*

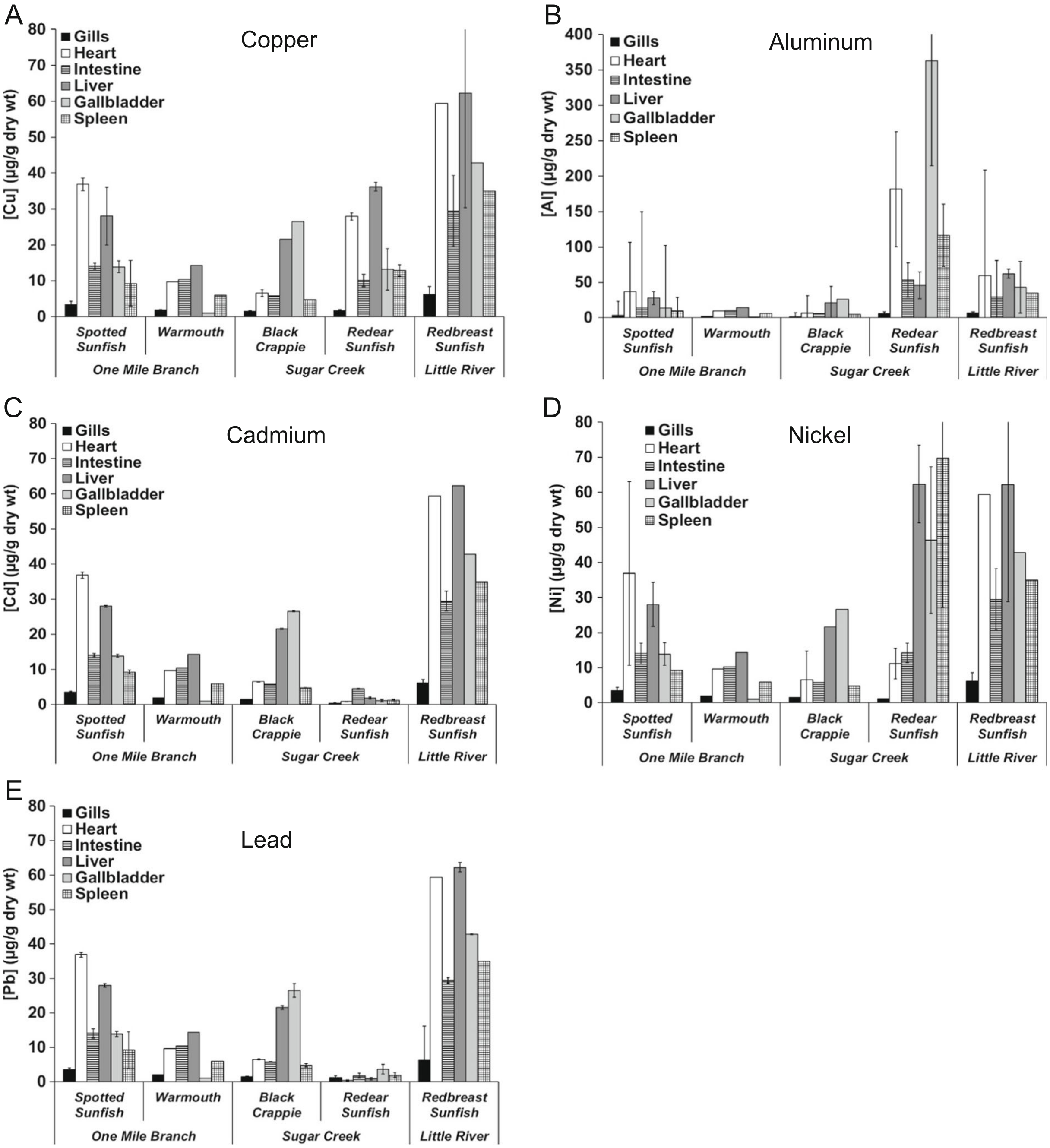

Fig. 5 Concentration (mean ± standard error) of **a** copper (Cu), **b** aluminum (Al), **c** nickel (Ni), **d** cadmium (Cd), and **e** lead (Pb) in the organs of large fish (9.0 to 15.0 cm) collected from two streams: One Mile Branch (*OMB*) and Sugar Creek (*SC*). The fish collected include *Lepomis punctatus* (spotted sunfish), *Lepomis gulosus* (warmouth), *Lepomis microlophus* (redear sunfish), *Pomoxis nigromaculatus* (black crappie), and *Lepomis auritus* (redbreast sunfish). $n = 2–7$ for each species

and *G. holbrooki* contained some of the most elevated tissue metal concentrations, and *G. holbrooki* was found at every site. It is possible that *G. holbrooki* is a more metal-tolerant species.

The metal distribution pattern in the organs of large fish was similar to those reported in other studies (Bielmyer et al. 2005; Vinodhini and Narayanan 2008) and those we have observed in our laboratory

Table 4 Quantification of biological diversity at each sampling site (One Mile Branch, OMB; Sugar Creek, SC; Little River, LR; and Withlacoochee, WIT)

Site	Shannon-Weiner's Index	Simpson's Index of Diversity
OMB	1.49	0.74
SC	1.61	0.77
LR	1.36	0.73
WIT	1.27	0.78

using the fish, *Fundulus heteroclitus* (Bielmyer et al. 2005, 2006; Bielmyer et al., unpublished). In freshwater, the primary binding sites for most waterborne metals are on the gills (Chowdhury et al. 2003; Bielmyer et al. 2005, 2006). The liver detoxifies excess metals from both the gill and intestine of teleost fish, and metals have also been reported to accumulate in this organ (Blanchard and Grosell 2005; Bielmyer et al. 2005, 2006). Bile is secreted by the liver and stored in the gall bladder, so metal may also pass into this organ, which is consitstent with our findings. Because different larger fish species were found in each of the streams, it is difficult to compare tissue metal concentrations across sites. The redbreast sunfish, which was found in LR, had the highest tissue metal concentrations; however, this could have been a consequence of the unique physiology of the fish rather than due to site differences. Another possibility of the higher tissue metal concentrations could be an unknown source of contamination.

Metals were observed in the water and three trophic levels of the streams studied; however, concentrations differed among sites, relating to differences in land use surrounding the rivers. Despite these differences, no significant changes in species biodiversity were observed among the four sites using either index (Table 3). These rivers were also found to be healthy using Beck's Index (Table 4). Macrophytes found and collected from the streams most impacted by human disturbance (OMB and SC) appeared to substantially accumulate and potentially remove metals from the water, as metal concentrations were most elevated in this trophic level. We did not, however, observe metal biomagnification up the food chain in this study. The observed metal removal (uptake into macrophytes) could have helped minimize effects on overall species biodiversity, thus protecting the aquatic communities in these systems. It should be noted that the unseasonable wet period could have confounded the impacts of the land use surrounding the sampling sites, and more research needs to be done in drier years to assess these endpoints. Additionally, downstream impacts of metals are not acknowledged in this paper. Lastly, the metal concentrations measured in the water column of these streams were near or below hardness-based U.S. EPA ambient water quality criterion values (USEPA 1984, 1986, 1987, 2007, 2016), yet metal accumulation was still observed in the biota. Standard stream health assessment measures, such as macroinvertebrate indices, would fail to detect biotic metal accumulation, as the presence of macroinvertebrates showed all streams to be healthy.

Table 5 Health of stream represented through macroinvertebrates, amount collected, and dominant collected groups at two sampling times (1 October and 2 December)

	Beck's Index	Individuals collected	Dominant groups
		Sampling 1	
OMB	8	79	Odonata, Hydrosychidae, Crayfish
SC	10	63	Hydrosychidae, Odonata, Coleoptera
LR	16	106	Clam, Megloptera, Ephemeroptera
WIT	9	38	Clam, Plecoptera, Crayfish
		Sampling 2	
OMB	9	136	Odonata, Megloptera
SC	10	54	Coleoptera, Odonata, Crayfish
LR	14	105	Clam, Coleoptera, Ephemeroptera
WIT	11	104	Clam, Crayfish, Odonata

The four streams include One Mile Branch, Sugar Creek, Little River, and Withlacoochi River

OMB One Mile Branch, *SC* Sugar Creek, *LR* Little River, *WIT* Withlacoochi River

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