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Spatial and taxonomic variation in trace element bioaccumulation in two herbivores from a coal combustion waste contaminated stream

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ABSTRACT

Dissimilarities in habitat use, feeding habits, life histories, and physiology can result in syntopic aquatic taxa of similar trophic position bioaccumulating trace elements in vastly different patterns. We compared bioaccumulation in a clam, *Corbicula fluminea* and mayfly nymph *Maccaffertium modestum* from a coal combustion waste contaminated stream. Collection sites differed in distance to contaminant sources, incision, floodplain activity, and sources of flood event water and organic matter. Contaminants variably accumulated in both sediment and biofilm. Bioaccumulation differed between species and sites with *C. fluminea* accumulating higher concentrations of Hg, Cs, Sr, Se, As, Be, and Cu, but *M. modestum* higher Pb and V. Stable isotope analyses suggested both spatial and taxonomic differences in resource use with greater variability and overlap between species in the more physically disturbed site. The complex but essential interactions between organismal biology, divergence in resource use, and bioaccumulation as related to stream habitat requires further studies essential to understand impacts of metal pollution on stream systems.

1. Introduction

Over decades, coal fired power plants have deposited millions of tons of coal combustion waste (CCW) into surface impoundment ponds across the United States (Luther, 2010). Constituents of CCW include heavy metals and other elements that the Environmental Protection Agency has deemed a risk to human health and the environment (Luther, 2010; Rowe et al., 2002). Coal-fired power plants operating on the Savannah River Site (SRS), SC, USA for over 55 years resulted in substantial releases of CCW into wetlands and streams in some locations (Cherry et al., 2004; Hopkins et al., 1997; Rowe et al., 2002). Streams impacted by CCW put organisms at risk by direct exposure and through subsequent trophic or maternal transfer of elements (Hopkins et al., 2000, 2006; Rowe and Hopkins, 2003). Contaminant bioaccumulation and adverse effects of CCW on aquatic organisms has been well documented in and near ash basins adjacent to our study stream, Beaver Dam Creek (BDC) (Rowe et al., 2002).

Evaluating the bioaccumulation of contaminants in primary consumers can provide insight to the routes of contaminant entry into consumer food chains. For example, some contaminants accumulate to higher levels in herbivorous than predaceous insects and some fishes (Arribére et al., 2010; Farag et al., 1998; Goodyear and McNeill, 1999). Additionally, extensive work on biodynamic models have found great variability in bioaccumulation of contaminants among aquatic insects; uptake rates from solution and diet frequently vary, but patterns of accumulation are often driven by rates of efflux (Buchwalter et al., 2008; Luoma and Rainbow, 2005; Poteat et al., 2013; Rainbow, 2002). We compared two invertebrate herbivores differing markedly in anatomy, physiology, habitat use, life history, and feeding behavior. The Asiatic clam (*Corbicula fluminea*) is generally tolerant of contaminants (Doherty, 1990). This widespread, invasive species (Araujo et al., 1993; Atkinson et al., 2010; Hakenkamp and Palmer, 1999) invaded our study stream Beaver Dam Creek as early as 1977 (Britton and Fuller, 1979). *C. fluminea* can be exposed to contaminants in the substrate directly by burrowing shallowly in the substrate and during feeding. *C. fluminea* utilize a wider array of resources than native mussels by both filter feeding and deposit feeding from surface sediments (Atkinson et al., 2010; Hakenkamp et al., 2001). Because of their tolerance, often abundance, relatively widespread distribution, and ability to bioaccumulate contaminants, all important characteristics of a biomonitor (Hare and Tessier, 1998; Hare et al., 2008; Luoma et al., 2009), *C. fluminea* have frequently been used to monitor pollution (Doherty, 1990; Paller et al., 2004; Peltier et al., 2009).

In contrast to *C. fluminea*, *Maccaffertium modestum* rarely inhabits loose sediments. *M. modestum* is a dorsoventrally flattened

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mayfly nymph (Ephemeroptera) adapted to cling to and scrape biofilm or opportunistically collect particulate organic matter (POM) from the surface of submerged objects such as wood debris (Merritt et al., 2008). *M. modestum* is known to bioaccumulate metals and has been successfully used in contaminant toxicity testing (Diamond et al., 1992; Xie et al., 2009). Mayflies are generally more sensitive to disturbance than *C. fluminea*, and their diversity and abundance are often used as an indicator of aquatic system health (Lakly and McArthur, 2000; Lenat, 1993). Further, extensive work has indicated Heptageniid mayflies to be among the most sensitive aquatic insects to heavy metal pollution (e.g. Buchwalter et al., 2008; Clements, 1994; Clements et al., 2000; Kiffney and Clements, 1994). However, some mayfly taxa can be more tolerant to reductions in water quality (Alba-Tercedor et al., 1995; Lenat, 1993). With a potential range of 0–10, the average Tolerance Value (TV) among 50 North Carolina, U.S. mayfly species was 2.7, but 5.8 for *M. modestum* and 6.3 in *C. fluminea* (Lenat, 1993). Even within the heptageniids, TVs varied greatly. However, the TV takes into account a suite of stressors, so it does not necessarily reflect sensitivity to metals alone. A taxon must be at least moderately tolerant to contaminant stress to allow a broad range of accumulation and to be present in sufficient numbers to analyze in polluted systems (Hare et al., 2008; Poteat et al., 2013). Additionally, while abundances of many macroinvertebrates are seasonal, relatively high densities of mixed sizes of *M. modestum* are present year-round (Jacobi and Benke, 1991; Kondratieff and Voshell, 1980; Smock, 1988). Recent taxonomic work has indicated the wide spread *M. modestum* to be a species complex (Webb et al., 2012). Consequently we acknowledge possible variability due to uncertain taxonomy of our specimens.

Here we compared trophic position ($\delta^{15}N$), carbon acquisition ($\delta^{13}C$) and trace element bioaccumulation of 16 elements between two herbivorous invertebrates inhabiting a CCW contaminated stream. Our study taxa have highly divergent habitat use and feeding behavior. Additionally, given the extreme differences in anatomy and physiology of these organisms, it would be surprising if modes and rates of uptake, detoxification and efflux did not differ between these taxa (Buchwalter et al., 2008; Luoma and Rainbow, 2005; Poteat et al., 2013; Rainbow, 2002). Here we use these organisms to investigate the bioavailability of contaminants in this system and begin to explore these organisms as biomonitors of CCW contaminated systems. Because the association of *C. fluminea* with the sediments and *M. modestum* to biofilm on wood debris, we additionally explored trace element accumulation in the sediments and biofilm. Environmental influences were further investigated by comparing samples of these two species collected at two sites differing in distance from contaminant sources, activity of the floodplain, and water sources during flood events.

2. Materials and methods

2.1. Study site descriptions

Invertebrates were collected from BDC, a Savannah River tributary, located on the SRS, a National Environmental Research Park operated by the Department of Energy along the southeast border of South Carolina, USA. The D Area coal-fired electric and steam generating power plant has been operated in the headwaters of BDC since the early 1950s (Halverson et al., 1997). The upper 2 km of BDC was channelized to transport large volumes of Savannah River water pumped through the powerhouse and effluents from the associated ash and coal pile runoff basins. Effluents flowed 6 km directly to the Savannah River during base flow, but moved as complex sheet flow through the Savannah River Swamp during flood events. Prior to construction of new basins, sluiced CCW overflowed from settling basins onto the Savannah River floodplain from the 1950s to early 1970s (Cherry and Guthrie, 1977, 1978). The resulting CCW overflow plume extends up to 2.7 m deep and over 40 ha (Roe et al., 2005) and is inundated when flooding of BDC and the Savannah River occur.

We selected two study sites in BDC that although only 430 m apart differ in hydrologic regimes. Our upper site, Site A, begins approximately 100 m above the Savannah River floodplain and extends 425 m upstream. The upper 165 m is channelized and the entire site deeply incised with an inactive floodplain. The Savannah River does not backup into this site during flood events, but it receives effluents from the powerhouse, ash basins, coal pile runoff basins as well as local runoff and groundwater. The downstream site, Site B, is a 350 m long segment of BDC on the Savannah River floodplain. At base flow, Site B shares the same water sources as Site A. However during flood events, Savannah River water flushes through the overflow ash plume area and inundates the less deeply incised Site B. On the day of water sampling, water quality parameters were measured in each site: Temperature (Site A=22.2, Site B=21.9), pH (Site A=7.08, Site B=7.01), conductivity (Site A=112, Site B=117 micromho), dissolved oxygen (Site A=90.3%, 7.56 mg/L, Site B=85.5%, 7.20 mg/L), and total dissolved solids (Site A=0.073, Site B=0.072 g/L).

2.2. Sample collection and handling

M. modestum and *C. fluminea* were collected and held in sieved BDC stream water for 24 h to allow gut evacuation (Peltier et al., 2009; Wilson et al., 2005). Specimens were rinsed in 18 MΩ deionized water prior to freezing. *C. fluminea* were later thawed and soft bodies removed for compositing. We collected five size classes (5 mm diameter ranges from 6 to 30 mm) of *C. fluminea*, however only three classes yielded sufficient composite masses for analyses: the 16–20 mm and 21–25 mm size classes at both sites and the 11–15 mm size class from Site A. Trace element and stable isotope analyses (SIA) were conducted on subsamples from the same composites. We obtained sufficient biomass such that a total of 13 *M. modestum* composite samples (Site A N=5; Site B N=8) and 19 *C. fluminea* composite samples (Site A N=11; Site B N=8) were analyzed.

A total of 33 sediment samples were collected and processed for trace element analyses and percent organic matter. From each site, eight samples were collected from swift runs with coarse sand representative of *C. fluminea* collection sites. An additional eight samples in Site B and nine from Site A were collected in each site from depositional zones where we expected highest contaminant loads (Paul and Meyer, 2001; Shelton and Capel, 1994). Five biofilm samples were harvested after 22–55 days from glass samplers placed in runs for an exploratory analysis. Biofilm sample sizes are presently inadequate to assess temporal or spatial variability, but provide a general estimate of contaminant levels accumulating in and on biofilms.

2.3. Trace element and stable isotope analyses

Trace element (Be, V, Cr, Ni, Cu, Zn, As, Se, Sr, Cd, Sb, Cs, Ba, Hg, Tl, and Pb) analysis was conducted on the invertebrates, 33 sediment samples, and five biofilm samples. Stable isotope (C and N) analysis was conducted on the invertebrates. Percent organic matter was determined for the sediment samples.

Samples were lyophilized, homogenized, and approximately 200–250 mg of dry sample was used for microwave digestion (MARS Xpress, CEM Corporation, Matthews, NC) with 10 ml trace metal-grade nitric acid (70% HNO_3). After separate digestion programs for invertebrates and sediment, samples were brought to final volumes of 15.0 ml and 50 ml respectively. A 1:10 dilution of the digested material was used for the trace element analysis performed by inductively coupled plasma mass spectroscopy (NexIon 300X ICP-MS; Perkin Elmer, Norwalk, CT). Mean instrument detection limits (ppb) for each element were as follows: Be (0.298), V (0.237), Cr (0.216), Ni (0.312), Cu (0.236), Zn (0.892), As (0.382), Se (1.28), Sr (0.270), Cd (0.264), Sb (1.42), Cs (0.463), Ba (0.509), Hg (0.148), Tl (0.478), and Pb (0.465). Certified reference material (LUTS-1 and TORT-2 for the invertebrates and MESS-3 for the sediment; National Research Council, Ottawa, ON, Canada) and blanks were included in the digestion and analysis for quality control purposes. Every tenth sample was processed as a duplicate for quality assurance. Mean percent recovery for elements in certified reference materials were as follows: V (100%), Cr (57%), Ni (78%), Cu (98%), Zn (91%), As (95%), Se (104%), Sr (92%), Cd (93%), and Hg (113%). Data were not corrected for percent recovery. All element concentrations are presented on a dry mass basis and presented as parts per million (ppm). Percent organic matter was determined from a subsample of each sediment sample by measuring the dry weight of the sediment and the ashed weight after four hours in a 490 °C muffle furnace.

Total C and N content and C and N stable isotope signatures were determined for the tissue samples with a Finnigan Delta Plus mass spectrometer (Thermo-Finnigan, Bremen, Germany). Isotope ratios were expressed in the delta (δ) format: $\delta^{13}C$ or $\delta^{15}N$ (units of ‰)$=(R_{sample}-R_{standard}/R_{standard})\times 1000$, where R is the ^{13}C:^{12}C ratio or ^{15}N:^{14}N ratio. A bovine standard was referenced against an international standard, and precision averaged $\leq 0.1\%$.

2.4. Statistical analyses

Concentrations of Sb and Tl were below detection limits (BDL) in $>96\%$ of the invertebrate samples and were excluded from further analyses. Additional high BDL rates were encountered in Be (78%), and Cs (84%) and these elements were excluded from parametric analyses. For statistical analyses, we replaced BDL concentrations