Recent precipitous declines of endangered freshwater mussels in the Clinch River: An *in situ* assessment of water quality stressors related to energy development and other land-use

A Final Completion Report

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Project Synopsis

The overall goal of this project was to evaluate the exposure and toxicological effects of contaminant stressors in the water and sediment of the Clinch River in areas of high mussel decline in Virginia and in areas of high abundance and recruitment in Virginia and Tennessee. The investigation also included an assessment of major tributary streams with varied histories of degradation, recovery and disturbance.

The specific objectives of this project were to:

1. Integrate existing data sets on mussel populations, NPDES discharges, mining and other energy permitted activities, pesticide use, and land use.

2. Using passive sampling devices, measure water concentrations of a suite of polar and non-polar organic contaminants, including current use pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), legacy organochlorine pesticides (OCPs), and natural and synthetic hormones at each site.

3. Measure water concentrations of inorganic contaminants (e.g., metals and metalloids), and nutrients (e.g., nitrate, nitrite, total ammonia nitrogen, total phosphorus) at each site.

4. Measure sediment concentrations (bed and pore water) of a suite of organic and inorganic contaminants, including current use pesticides, PAHs, PCBs, legacy OC pesticides, natural and synthetic hormones, metals and metalloids, nutrients (e.g., nitrate, nitrite, total ammonia nitrogen, total phosphorus), and total organic carbon at each site.

5. Conduct ASTM Method E 2455-06 toxicity tests with juvenile mussels using sediment pore water collected from each site.

6. Compare the results of the toxicity tests to measured concentrations of contaminants in surface and pore water and sediment compartments (objectives 2-4) from the study sites and to published criteria and toxicity benchmark values for standard aquatic test organisms and mussels.

7. Assess growth and survival of mussels *in situ* at each site over two years.

8. Synthesize the findings to inform management efforts for aquatic species and water quality in the Clinch River watershed and elsewhere.

All objectives have been completed and are reported upon in the following four Chapters.

EXECUTIVE SUMMARY

Freshwater mussels (Order Unionoida) are one of the most rapidly declining faunal groups in North America. Numerous causes include habitat destruction, invasive species, economic exploitation, and degradation of water quality with increasing human encroachment and development. The Clinch River watershed of Virginia and Tennessee formerly supported one of the nation's greatest concentrations of freshwater mussel biodiversity, but agricultural and mining practices, land development, contaminant spills, and other anthropogenic activities have degraded water and sediment quality in the Virginia section. Mussel populations in certain reaches have declined in both species richness and abundance, and there was a critical need to investigate the effects of potential landscape and chemical alterations to the system. In Chapter 1, we assessed landscape changes from 2001to 2011 within the Clinch River watershed, the four main tributary watersheds, and specifically the mainstem reach of the Clinch River in Virginia identified as a mussel zone of decline (ZOD). We found that there was an 8.2% decrease in forest cover in the Clinch River watershed with a corresponding increase of barren land (identified as active and recent surface mining areas) and scrub-shrub cover types of 197% over the assessment period. Developed land cover and impervious surface area also increased 39.7% and 4.7%, respectively. There are approximately 26,902 acres of permitted surface mining operations in the Clinch River watershed in Virginia. Moreover, permitted oil and gas well operations are present that affect water quality within the mussel ZOD and a majority of those wells are located in the Guest River tributary that enters the Clinch River immediately upstream of the ZOD. The aim of Chapter 2 was to evaluate the exposure of adult resident mussels to a suite of contaminant stressors throughout the Clinch River to provide insight into the potential role of contaminants in the decline of mussels in the mainstem Clinch River (8 sites) and its tributaries (4 sites). We quantified and related inorganic and organic contaminant concentrations in mussels to their associated habitat compartments (bed sediment, particulate sediment, pore water, and surface water) and found that metals in resident mussels were, for the most part, unrelated to the spatial pattern of variation of metals in all four habitat compartments. However, concentrations of organic contaminants, particularly the polycyclic aromatic hydrocarbons (PAHs) in resident mussels were strongly related to PAH concentrations in surface water, pore water, particulate and bed sediment and the spatial pattern in mussel densities within the river and in the ZOD. This portion of the project revealed that PAHs are an important source of contamination in the Clinch River. In Chapter 3, we address the effects of inorganic and organic contaminant exposure on the growth and survival of juvenile freshwater mussels (*Villosa iris* in 2012 and *Lampsilis fasciola* in 2013) deployed at the 12 sites in the Clinch River and its tributaries. We used two *in situ* deployment techniques; cages filled with ambient stream bed sediment and enclosures (silos) positioned at the sediment-water interface. In the two consecutive years of the study, we found that over each 5-6 month deployment period, mussel survival was generally high at most sites, but that growth rate varied by site and was negatively correlated with tissue concentrations of certain mining-associated metals, especially in caged mussels in 2012. Growth rate also differed between deployment methods and was considerably greater for juvenile mussels in cages than in silos between years. Substantial amounts of PAHs accumulated in the juvenile mussels over the relatively brief deployment period and were similar to concentrations measured in resident mussels from Chapter 2. Moreover, PAH concentrations in juvenile mussels were strongly correlated with native mussel density, but not with metal tissue concentrations. These findings suggest that PAHs may exert chronic, potentially lethal effects

on mussels, whereas certain metals, when present at high enough concentrations, can have sublethal effects through depressed growth. Chapter 4 examined the toxicity of recently-deposited sediments on juveniles of the freshwater mussel *Epioblasma brevidens*, by collecting time-integrated sediment samples from the water column with sediment traps from 11 sites in the Clinch River Basin, including 7 sites within the ZOD. Sediment samples were analyzed to identify presence and concentration of metals and organic contaminants. Mussels were exposed to the riverine sediments and to three control sediments for 28 d; survival, shell length, and biomass were then assessed. Sediment treatment (i.e., river location) had a significant effect on mussel survival ($p < 0.01$) and biomass ($p = 0.02$), but did not affect length ($p = 0.37$), and sediments from two of the tributaries were the most toxic. Metals and polycyclic aromatic hydrocarbons were prevalent in sediment samples collected from all sites. Manganese was significantly correlated with mussel survival and biomass, as was ammonia with survival, and total organic carbon with biomass. The landscape analysis of potential contaminant sources identified in Chapter 1 indicate mining and agriculture likely contributed to elevated manganese and ammonia, respectively. Sediments collected with sediment traps over relatively short durations of deployment can help elucidate recent contaminant influx and its potential for inducing toxicity in mussels. In conclusion, our multi-year field and laboratory study provided new information on the potential role of contaminants in freshwater mussel declines within the Clinch River ecosystem. We identified PAHs as the major organic contaminant of concern in this system. Moreover, our results demonstrated a clear link between PAH body burdens and mussel health given the strong negative correlations between site-specific mussel density and PAH tissue concentrations measured in both juveniles and adults. While PAHs appear to have a potentially chronic lethal effect on mussel populations in the Clinch River, metals seem to exert more subtle sub-lethal effects on growth, at least in years when metals reach concentrations high enough in mussels to elicit negative effects. Our identification of the PAH class of organic contaminants and individual metals of concern will aid management and conservation efforts by guiding future studies of the likely sources of those specific contaminants and assist in identifying contaminants to focus on for future laboratory toxicity studies.

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Chapter 1: Clinch River Watershed Landscape-Level Geospatial Analyses

Geospatial landuse analyses were performed for the Clinch River watershed in Virginia and Tennessee (ArcGIS v10.1) to assess changes in land cover types. Geographical and temporal trends within sub-watersheds were also determined. Landcover change for the Clinch River Watershed was derived from the Multi-Resolution Land Characteristics Consortium (MRLC) National Landcover Database (NLCD). The NLCD serves as the definitive Landsat-based, 30-meter resolution, land cover database for the Nation. NLCD provides spatial reference and descriptive data for characteristics of the land surface such as thematic class (e.g., urban, agriculture, and forest), percent impervious surface, and percent tree canopy cover. NLCD supports a wide variety of Federal, State, local, and nongovernmental applications that seek to assess ecosystem status and health, understand the spatial patterns of biodiversity, predict effects of climate change, and develop land management policy. NLCD products are created by the MRLC Consortium, a partnership of Federal agencies. Landcover change layers for the Clinch River watershed were created from NLCD change layers from 2001-2006 and 2006-2011 (Table 1). Data layers for impervious surfaces were also acquired for the Clinch River watershed for the same areas.

Table 1. Landuse Analyses Data Layers

Boundaries: • **Counties** • **HUC 8 watersheds** • **City limits** **Project Sample Sites:** • **Mainstem sites** • **Tributary sites** **EPA/Virginia Data:** • **NPDES** • **EPA geodata** **Infrastructure:** • **Roads** **Public Lands:** • **Protected Areas Database (PAD)** **Mineral Extraction Data:** • **Oil and Gas wells** • **Surface mines** • **Manufacturing mines** • **Miscellaneous mineral operations** • **Non-Ferrous metal mines** • **Crushed stone mines**	**Hydrography:** • USGS NHD flowlines • Hydrography junctions • Catchments **Landuse/Landcover:** • NLCD Landcover 2001 • NLCD Landcover 2006 • NLCD Landcover 2011 • NLCD Landcover change 1992-2001 • NLCD Landcover change 2001-2006 • NLCD Landcover change 2006-2011 • NLCD Impervious surface 2001 • NLCD Impervious surface 2006 • NLCD Impervious surface 2011

Acreages for landcover change (Table 2) in the Clinch River watershed were calculated for each landcover type using the formula (count * 900)/4046.86. Count being the number of 30 meter cells for each landcover type, multiplied by the number of square meters per cell (900) and divided by the number of square meters per acre (4046.86).

Open water landcover is characterized as all areas of open water; typically 25 percent or greater cover of water per pixel. Developed area landcover types are characterized by a high percentage (30 percent or greater) of constructed materials (e.g. asphalt, concrete, buildings, etc). Low-intensity residential landcover includes areas with a mixture of constructed materials and vegetation. Constructed materials account for 30-80 percent of the cover. Vegetation may account for 20 to 70 percent of the cover. These areas most commonly include single-family housing units. High-intensity residential includes highly developed areas where people reside in high numbers. Examples include apartment complexes and row houses. Vegetation accounts for less than 20 percent of the cover. Constructed materials account for 80 to100 percent of the cover.

Barren areas are characterized by bare rock, gravel, sand, silt, clay, or other earthen material, with little or no "green" vegetation present regardless of its inherent ability to support life. Vegetation, if present, is more widely spaced and scrubby than that in the "green" vegetated categories; lichen cover may be extensive. These barren areas include quarries, strip mines, and gravel pits; however, for the purposes of this analysis, permitted surface mining area data from the State of Virginia were utilized and do not necessarily reflect “active” mining.

Vegetation cover types are classified as Deciduous Forest, Evergreen Forest, Mixed Forests, Scrub-Shrub, Herbaceous, Hay/Pasture, Cultivated Crops, or Woody Wetlands. Deciduous Forest - Areas dominated by trees where 75 percent or more of the tree species shed foliage simultaneously in response to seasonal change. Evergreen Forest - Areas dominated by trees where 75 percent or more of the tree species maintain their leaves all year. Canopy is never without green foliage. Mixed Forest - Areas dominated by trees where neither deciduous nor evergreen species represent more than 75 percent of the cover present. Woody Wetlands - Areas where forest or shrubland vegetation accounts for 25-100 percent of the cover and the soil or substrate is periodically saturated with or covered with water.

Scrub/shrub landcover is characterized by natural or semi-natural woody vegetation with aerial stems, generally less than 6 meters tall, with individuals or clumps not touching to interlocking. Both evergreen and deciduous species of true shrubs, young trees, and trees or shrubs that are small or stunted because of environmental conditions are included. Scrub/shrub landcover is also dominated by shrubs; shrub canopy accounts for 25-100 percent of the cover. Shrub cover is generally greater than 25 percent when tree cover is less than 25 percent. Shrub cover may be less than 25 percent in cases when the cover of other life forms (e.g. herbaceous or tree) is less than 25 percent and shrubs cover exceeds the cover of the other life forms.

Grasslands/herbaceous landcover types are areas dominated by upland grasses and forbs. In rare cases, herbaceous cover is less than 25 percent, but exceeds the combined cover of the woody species present. These areas are not subject to intensive management, but they are often utilized

for grazing. Planted/cultivated landcover types are areas characterized by herbaceous vegetation that has been planted or is intensively managed for the production of food, feed, or fiber; or is maintained in developed settings for specific purposes. Herbaceous vegetation accounts for 75-100 percent of the cover. Pasture/hay landcover types are grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops. Row crop landcover type are areas used for the production of crops, such as corn, soybeans, vegetables, tobacco, and cotton.

Impervious surfaces are mainly artificial structures, such as pavements (roads, sidewalks, driveways and parking lots), that are covered by impenetrable materials such as asphalt, concrete, brick, stone, and rooftops. The actual degree of imperviousness is not explicitly defined.

Topographic contour intersects for streams at 368' – 1154' MSL and paved roads at 371' to 1271' MSL were performed. All paved roads within the Zone of Decline (ZOD) were extracted from the streets spatial data layer (Navteq 2015) using the clip geoprocessing tool in ArcGIS. The clip tool uses a boundary to capture only desired spatial information within the boundary.

Table 2. Clinch River Watershed (Hydrologic Unit 06010205) NLCD

LAND COVER Type	2001 ACRES	2006 ACRES	2011 ACRES	Acreage Change from 2001-2011	Percent Change
Open Water	35117.45	35170.82	35191.73	74.28	0.20
Developed, Open Space	103419.74	103267.18	105228.47	1808.73	1.70
Developed, Low Intensity	53980.07	55547.07	56321.67	2341.6	4.30
Developed, Medium Intensity	18803.47	19590.08	22465.86	3662.39	19.50
Developed, High Intensity	4572.66	4531.74	5297.44	724.78	15.90
Barren Land	6733.67	7469.57	8128.97	1395.3	20.70
Deciduous Forest	918779.45	910937.81	904880.23	-14899.42	-1.50
Evergreen Forest	46924.83	45298.23	45358.72	-1566.11	-3.50
Mixed Forest	60251.38	58598.10	58365.92	-1885.46	-3.20
Shrub/Scrub	4606.02	6031.79	12742.32	8136.3	177.00
Herbaceuous	112140.05	120503.87	116289.05	4149	3.70
Hay/Pasture	289616.76	288352.01	284965.60	-44651.16	1.60
Cultivated Crops	1635.27	1536.97	1483.37	-151.9	10.20
Woody Wetlands	6979.41	6721.88	6516.83	-462.58	7.10
Emergent Herbaceuous Wetlands	17.12	20.24	341.15	324.03	1800.80
Impervious Surface	181433.56	188467.01	190024.88	8591.32	4.7

There was an 8.2% decrease in forest cover in the Clinch River watershed with a corresponding increase of barren land and scrub-shrub cover types of 197% over the assessment periods of 2001 to 2011. Developed land cover and impervious surface area also increased 39.7% and 4.7%, respectively.

According to 2006 Virginia Department of Mines and Minerals Enforcement data, there are approximately 26,902 acres of permitted surface mining operations in the Clinch River watershed in Virginia. Active and recent surface mining areas are classified as Barren land cover in the NLCD. Numerous permitted oil and gas well operations are also present that may affect water quality within the Zone of Decline (ZOD) with a majority of those wells located in the Guest River catchment.

Guest River

The Guest River flows for nearly its entire length in Wise County. The catchment rises in the north-central part of the county and flows initially south-southeastwardly to the city of Norton; then eastwardly to the town of Coeburn; then southeastwardly for the remainder of its course. It flows into the Clinch River near According to the NLCD, this watershed comprises approximately 62,000 acres (Table 3). There are significant development and mineral extraction operations in the watershed. The incorporated towns of Norton and Wise are also in the watershed.

Table 3. Guest River Catchment NLCD.

LAND_COVER	2001 ACRES	2006 ACRES	2011 ACRES	Acreage Change	Percent Change
Open Water	181.696	143.889	149.227	-32.470	-17.87
Developed, Open Space	3231.839	3463.129	3313.458	81.619	2.53
Developed, Low Intensity	3649.941	3655.056	3772.925	122.984	3.37
Developed, Medium Intensity	1893.246	1901.697	2080.280	187.034	9.88
Developed, High Intensity	332.925	342.710	394.306	61.381	18.44
Barren Land	1755.139	1701.764	1732.677	-22.462	-1.28
Deciduous Forest	38875.919	39187.271	39162.585	286.667	0.74
Evergreen Forest	2086.729	1943.284	1943.284	-143.445	-6.87
Mixed Forest	1020.124	974.533	993.437	-26.687	-2.62
Shrub/Scrub	0.000	132.770	114.756	-18.014	-13.57
Herbaceuous	3147.774	3097.957	3024.567	-123.207	-0.04
Hay/Pasture	7938.377	7549.186	7411.746	-526.631	-0.07
Cultivated Crops	20.460	20.460	20.460	0.000	0.00
Woody Wetlands	0.000	20.460	20.460	0.000	0.00
Impervious Surface	9139.09	9555.52	9587.43	448.34	4.91

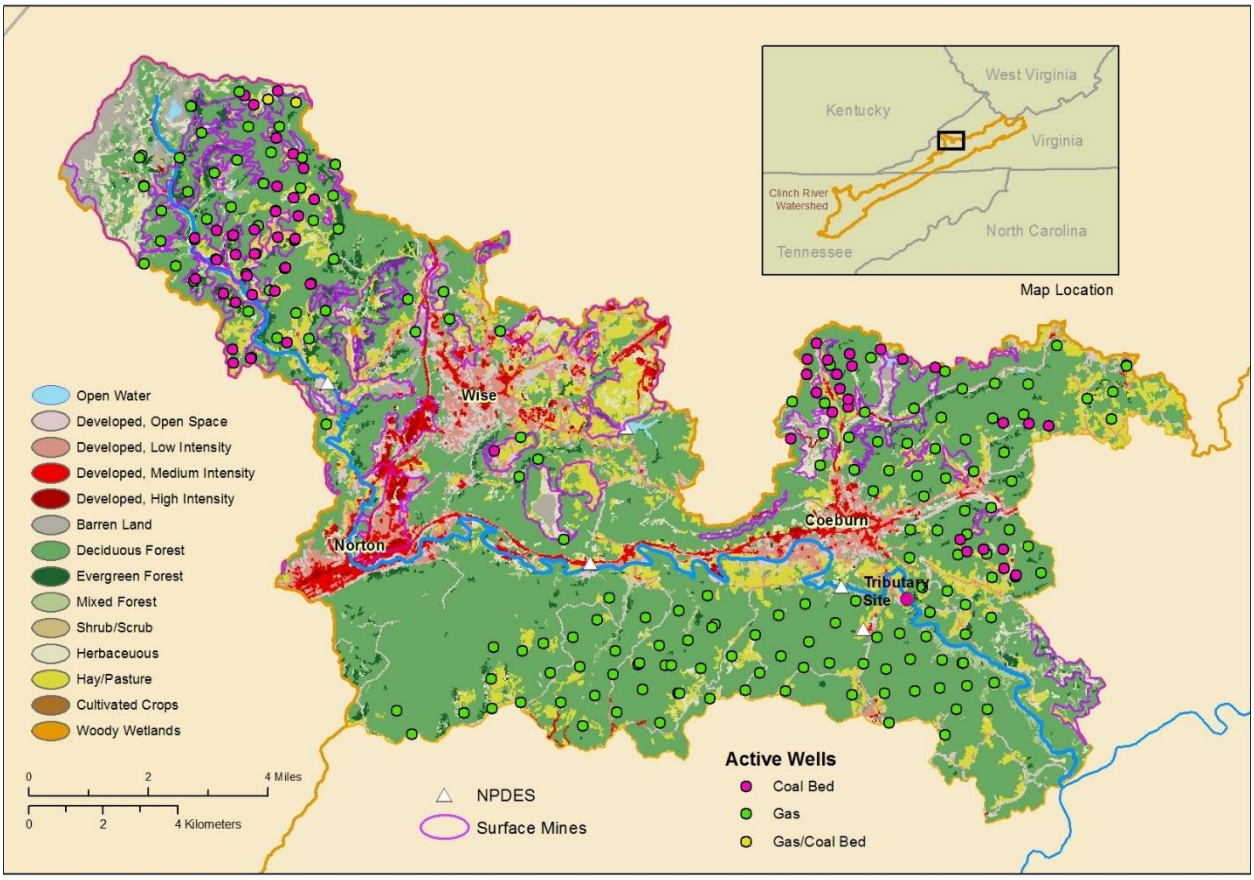

Figure 1. Guest River Watershed

Dumps Creek

The headwaters of the Dumps Creek catchment originate in Russell County. This sub-watershed drainage area comprises approximately 121,000 acres (Table 4) and joins the Clinch River at Carbo, Virginia.

Surface mining operations in the watershed comprise over 8,000 acres, including active surface mining and reclaimed areas. There are also numerous abandoned historical mining areas (i.e., exposed high walls, spoil piles/tailings ponds).

Table 4. Dumps Creek Catchment NLCD

LAND_COVER	2001 ACRES	2006 ACRES	2011 ACRES	Acreage Change	Percent Change
Open Water	456.799	459.245	489.046	32.247	6.59
Developed, Open Space	6438.770	4545.302	6250.179	-188.591	-3.02
Developed, Low Intensity	2608.244	3362.829	2632.485	24.241	0.92
Developed, Medium Intensity	781.495	896.250	926.051	144.557	15.61
Developed, High Intensity	62.048	91.182	79.395	17.347	21.85
Barren Land	935.392	773.044	1104.189	168.798	15.29
Deciduous Forest	75234.775	75368.656	75086.882	-147.892	-0.20
Evergreen Forest	1993.101	1943.062	1900.140	-92.961	-4.89
Mixed Forest	1060.822	1039.028	1027.908	-32.914	-3.20
Shrub/Scrub	45.146	129.656	198.821	153.675	77.29
Herbaceuous	6548.855	7524.500	7207.810	658.955	9.14
Hay/Pasture	24805.899	24828.583	24041.751	-764.148	-3.18
Cultivated Crops	163.905	163.905	171.244	7.339	4.29
Woody Wetlands		10.008	10.230	10.230	
Emergent Herbaceous Wetlands			9.118	9.118	
Impervious Surface	9917.69	9917.69	9918.58	0.89	0.01

There was an increase in developed land cover types of approximately 39% over the assessment periods. Barren land and scrub-shrub land cover types increased significantly (92%) and may be indicative of active surface mining operations (Figure 2).

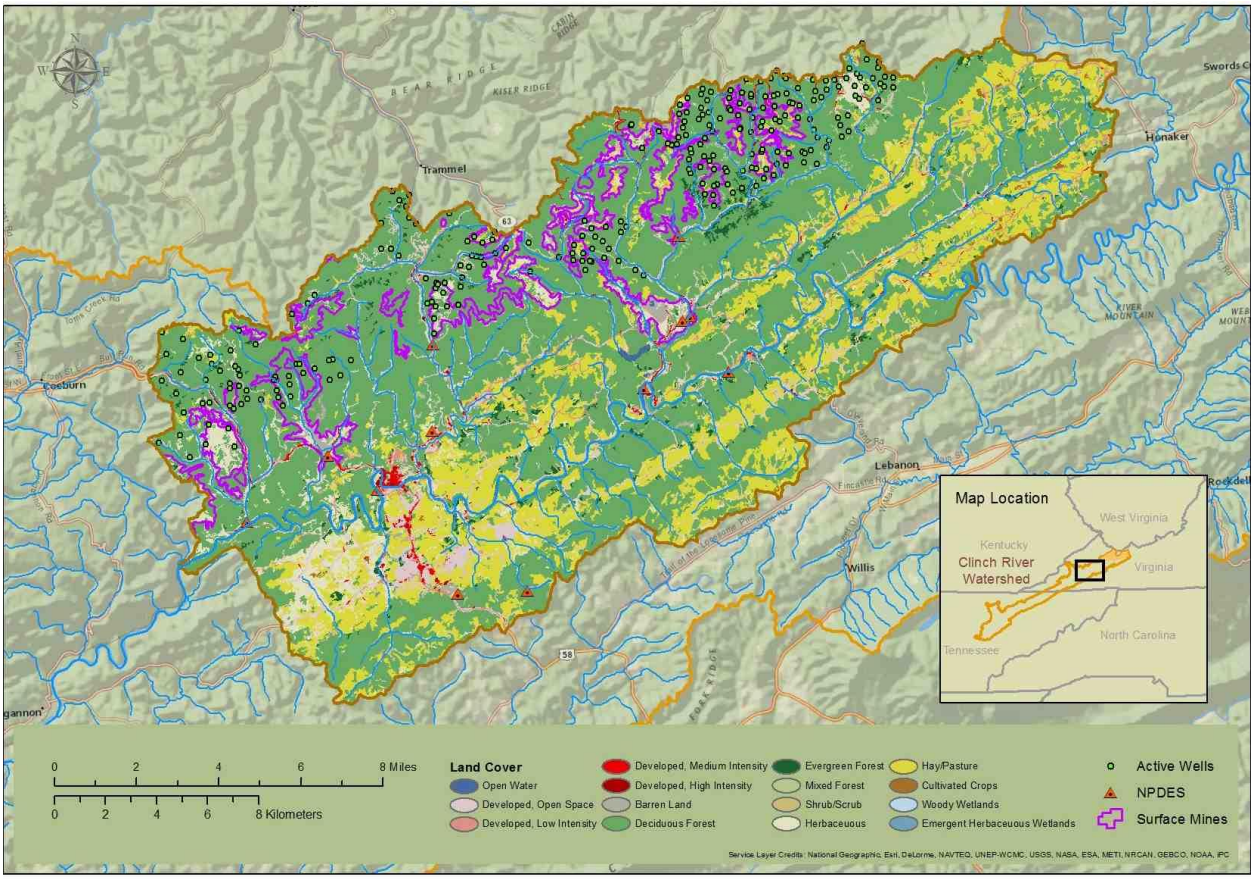

Figure 2. Dumps Creek Watershed

Indian Creek

The headwaters of the Indian Creek catchment originate in the primarily forested northernmost part of Tazewell County. Indian Creek flows to the southwest, where it joins the Clinch River near Cedar Bluff, Virginia. This sub-watershed drainage area comprises approximately 144,000 acres (Table 5).

Table 5. Indian Creek Catchment NLCD

LAND_COVER	2001 ACRES	2006 ACRES	2011 ACRES	Acreage Change	Percent Change
Open Water	84.065	84.065	87.623	3.558	4.06
Developed, Open Space	6850.645	5872.998	6776.365	-74.280	-1.10
Developed, Low Intensity	5348.146	5685.519	5366.160	18.014	0.34
Developed, Medium Intensity	2809.289	3049.920	2898.914	89.625	3.09
Developed, High Intensity	374.957	347.158	395.195	20.238	5.12
Barren Land	544.200	914.042	983.874	439.674	44.69
Deciduous Forest	89452.464	89106.863	88883.356	-569.108	-0.64
Evergreen Forest	6270.862	6225.049	6220.378	-50.484	-0.81
Mixed Forest	708.105	702.100	702.767	-5.337	-0.76
Shrub/Scrub	96.074	96.074	96.074	0.000	0.00
Herbaceous	4910.029	5285.654	5322.571	412.542	7.75
Hay/Pasture	25820.241	25900.081	25535.798	-284.443	-1.11
Cultivated Crops	162.570	162.126	162.570	0.000	0.00
Impervious Surface	15402.16	15455.32	15456.87	54.71	0.36

Developed land cover types increased 8.55% over the assessment periods with minimal loss of forested land cover types (Figure 3); however, the barren land cover type increased approximately 45%.

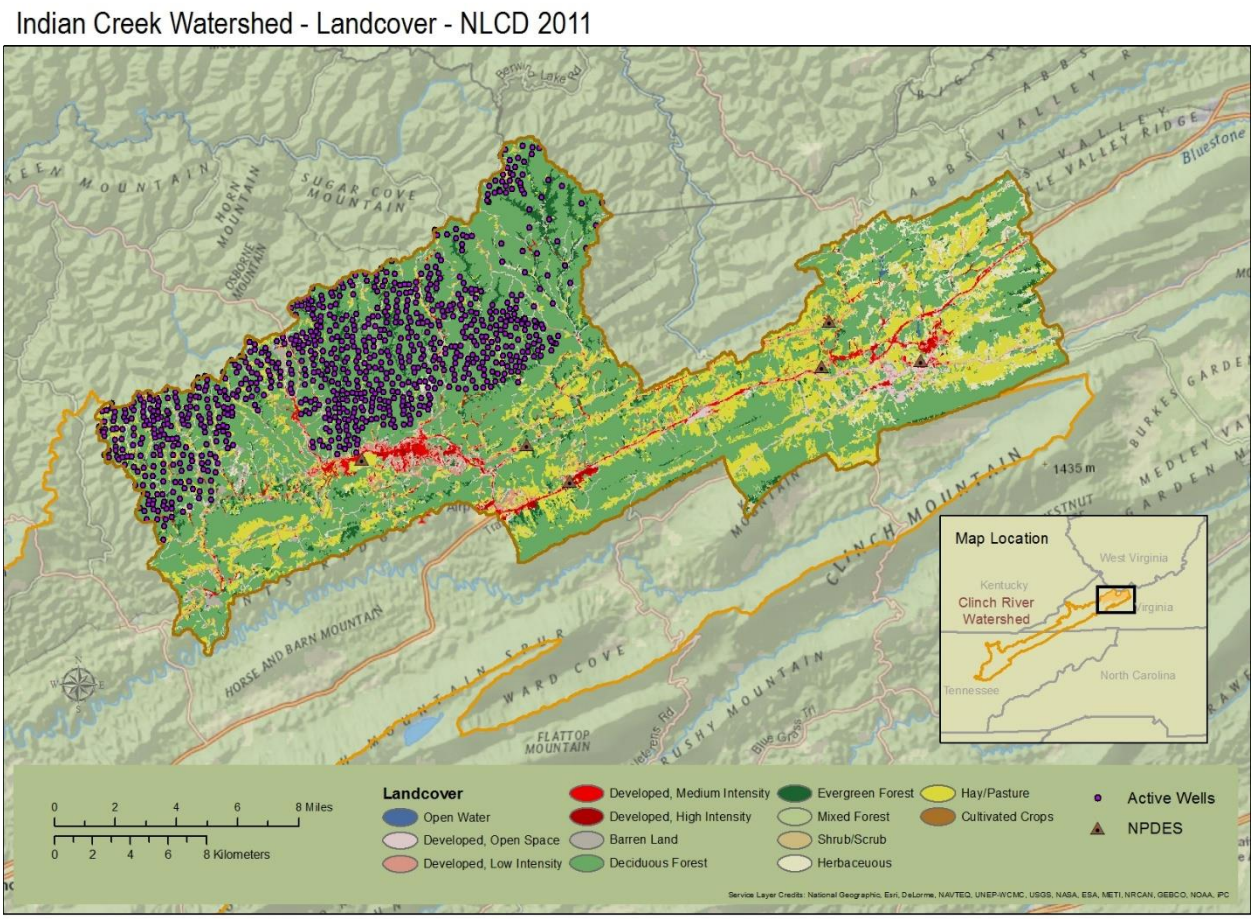

Figure 3. Indian Creek Watershed

Copper Creek

The headwaters of the Copper Creek catchment originate in Russell County River. It flows into the Clinch River near Speers Ferry, Scott County. The sub-watershed drainage area is approximately 85,000 acres. It is primarily rural with significant agricultural operations (e.g., grazing) occurring in the watershed (Table 6).

Table 6. Copper Creek Catchment NLCD

LAND_COVER	2001 ACRES	2006 ACRES	2011 ACRES	Acreage Change	Percent Change
Developed, Open Space	4157.446	4185.467	4124.754	-32.692	-0.79
Developed, Low Intensity	252.863	250.416	259.312	6.449	2.49
Developed, Medium Intensity	12.676	12.676	38.474	25.798	67.05
Barren Land	151.451	136.995	129.878	-21.572	-16.61
Deciduous Forest	41677.424	41535.536	40703.558	-973.866	-2.39
Evergreen Forest	985.208	980.093	961.412	-23.796	-2.48
Mixed Forest	930.944	910.484	894.026	-36.918	-4.13
Shrub/Scrub	642.498	639.385	1242.519	600.021	48.29
Herbaceuous	1720.445	1855.661	2126.093	405.648	19.08
Hay/Pasture	34760.506	34784.747	34811.434	50.928	0.15
Cultivated Crops	68.942	68.942	68.942	0.000	0.00
Woody Wetlands	7.117	7.117	7.117	0.000	0.00
Impervious Surface	4538.85	4538.85	4538.85	0	0.00

There was a 9.0% decrease in all forest land cover types in the watershed during the assessment periods. No changes in agricultural land cover types and impervious surfaces were observed (Figure 4).

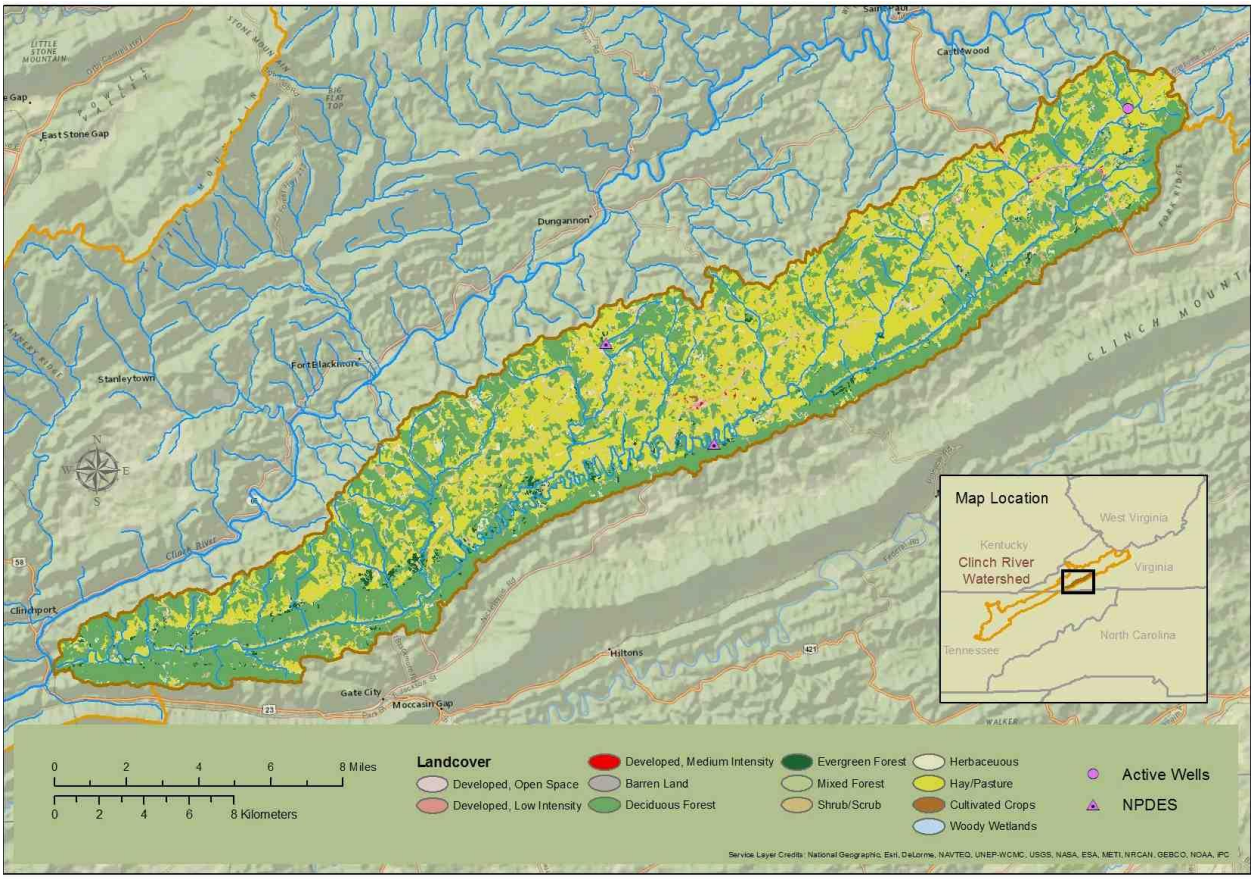

Figure 4. Copper Creek Watershed

Zone of Mussel Decline

The incorporated communities of Richlands, Cleveland, and St. Paul lie on the mainstem Clinch River within the ZOD. There are approximately 350,000 acres of watershed drainage area influencing this focus area (Table 7). Developed land cover and impervious surface increased 37.8% over the assessment periods with a corresponding loss of all forest land cover types of 9.2%. There are approximately 551 miles of paved roads below 1271' MSL.

Approximately 518 miles of the roads within 100 feet of streams were classified as basic two lane roads. Using the U.S Department of Transportation Federal Highway Administration Interstate road minimum lane width of 12 feet as a basic guide and excluding any added paved shoulder area as an accommodation for local roads that do not meet the interstate highway minimum road width design standard, 518 miles x 5280 (feet per mile) x 24 (approximate width of a 2 lane street) = 65,640,960 sq feet / 43560 (sq ft in an acre) = 1,506.9 acres of paved two-lane roads (impervious surface). Approximately 33 miles of roads within the ZOD were classified as four-lane highways. Using the same formula and doubling the width, 192 acres of paved four lane highways were calculated, created a total of 1,698.9 acres of impervious surface classified as paved roads within 100 feet of streams in the ZOD.

Table 7. Clinch River Zone of Mussel Decline NLCD

LAND COVER TYPE	2001 ACRES	2006 ACRES	2011 ACRES	Acreage Change	Percent Change
Open Water	799.51	771.71	806.63	7.12	0.8
Developed, Open Space	17304.08	15349.68	17153.97	-150.11	-0.8
Developed, Low Intensity	5654.83	6474.80	5811.39	156.56	2.7
Developed, Medium Intensity	2620.48	2756.58	2986.09	365.61	13.9
Developed, High Intensity	395.86	434.11	473.03	77.17	19.5
Barren Land	2860.22	2630.04	2997.44	137.22	4.8
Deciduous Forest	222929.73	223569.11	221111.87	-1817.86	-0.8
Evergreen Forest	7058.36	6778.81	6728.77	-329.62	-4.7
Mixed Forest	4251.07	4128.09	4094.95	-156.12	-3.7
Shrub/Scrub	1236.74	1429.78	3337.03	2100.29	170.0
Herbaceous	13949.93	15056.12	14752.77	802.84	5.7
Hay/Pasture	71732.50	71389.57	70476.86	-1255.64	-1.7

Cultivated Crops	456.35	450.57	450.79	-5.56	-1.0
Woody Wetlands	290.00	320.69	311.13	21.13	7.2
Emergent Herbaceous Wetlands			46.93		
Impervious Surface	26265.47	26680.02	26717.38	451.91	1.72

Utilizing available agency data, sources of contaminants of potential environmental concern (COPECs) influencing the native mussel fauna were evaluated. There are five (5) major and numerous minor wastewater and stormwater discharges in the Clinch River watershed (Table 8) that could potentially affect aquatic resources within the Zone of Decline. Older EPA NPDES data were reconciled with more current State of Virginia data for individual permits (Table 9). Discharges from treatment systems treating domestic waste with a design flow greater than 1.0 MGD or with a pre-treatment program are classified as major discharges. Industrial and commercial discharges are classified based on several factors including flow, waste characteristics and water quality and health impacts. Approximately, 300 active oil and gas operations and 27,000 acres of permitted surface mining operations influence water quality within the Zone of Decline (Figure 5).

Table 8. Point and Non-point Sources for COPECs

Watershed	**Guest River**	**Indian Creek**	**Copper Creek**	**Dumps Creek**	**Zone of Decline**
NPDES Permits	5	8	2	12	23
Oil and Gas Wells	182	43	1	71	293
Total Permitted Mining Acres	10,765.5	5,082.4	0	8,073.6	26,902

Table 9. NPDES Permits Influencing Zone of Mussel Decline

FACILITY NAME	LOCATION	PERMIT NUMBER	MAJOR/MINOR DISCHARGE
DUFFIELD INDUSTRIAL PARK WWTP	DUFFIELD	VA0029564	MINOR
DANTE COMMUNITY WWTP	DANTE	VA0088935	MINOR
DUNGANNON STP	DUNGANNON	VA0070670	MINOR
C-N-W REGIONAL WWTP	COEBURN	VA0077828	MAJOR
AMERICAN ELECTRIC POWER - CLINCH RIVER PLANT	CLEVELAND	VA0001015	MAJOR
DICKENSON RUSSELL COAL CO - MOSS 3 PREP PLANT STP	CLEVELAND	VA0065951	MINOR
DICKENSON RUSSELL COAL CO – LAUREL MOUNTAIN MINE	CLEVELAND	VA0085278	MINOR
DOMINION VIRGINIA CITY	ST. PAUL	VA0092746	MAJOR
SAINT PAUL WWTP	ST. PAUL	VA0026221	MINOR
RICHLANDS REGIONAL WWTF	RICHLANDS	VA0021199	MAJOR
TAZEWELL WWTP	TAZEWELL	VA0026298	MAJOR
NICKELSVILLE WWTP	NICKELSVILLE	VA0087955	MINOR

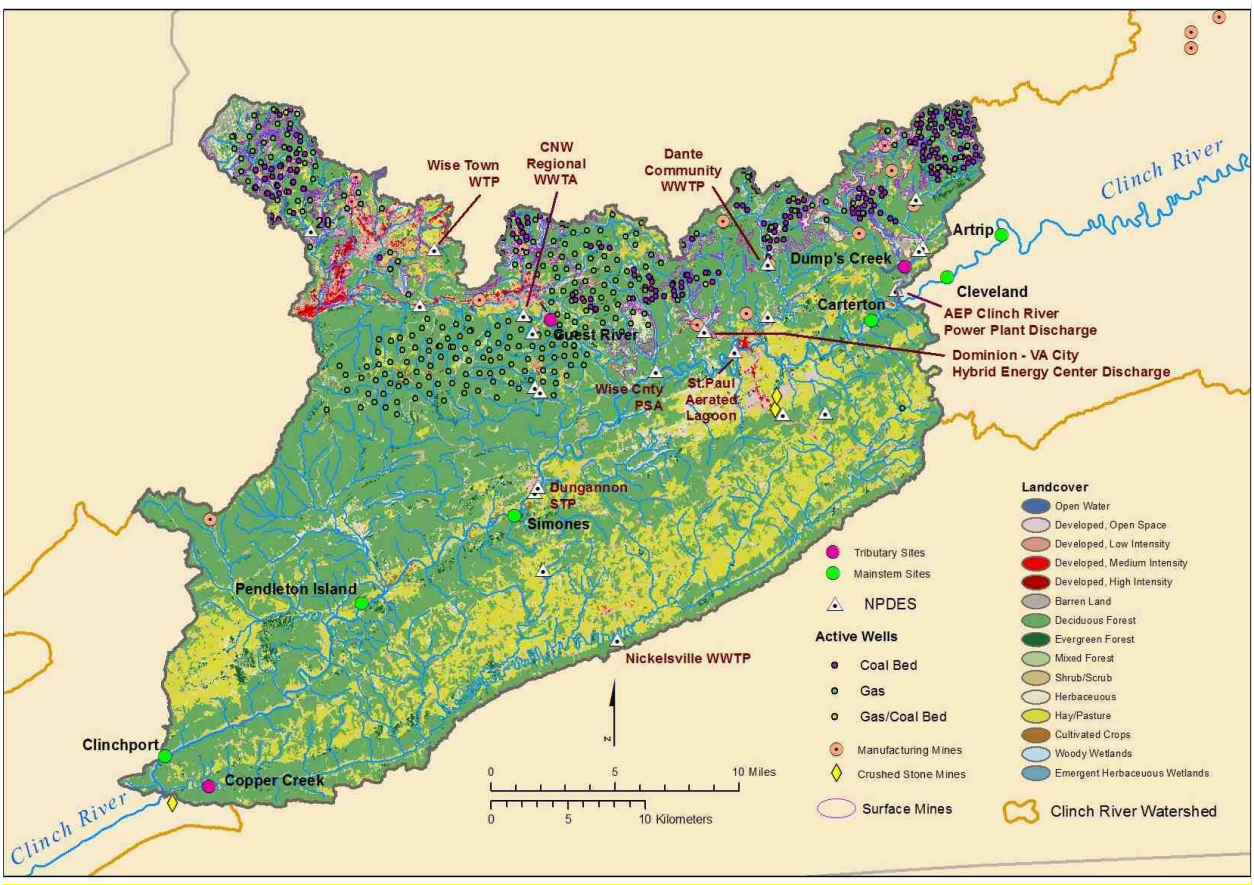

Figure 5. Clinch River Zone of Mussel Decline

Chapter 2: Assessment of inorganic and organic contaminants in native freshwater mussels and their habitats in the Clinch River, USA

Abstract

The Clinch River watershed of the upper Tennessee River basin of Virginia and Tennessee supports one of North America's greatest concentrations of freshwater biodiversity, including 46 extant species of native freshwater mussels (Order Unionoida), 20 of which are protected as federally endangered. Despite the global biological significance of the Clinch River, mussel populations are declining in some reaches, both in species richness and abundance. The aim of this study was to evaluate the exposure of adult resident mussels to a suite of inorganic and organic contaminant stressors throughout a region of the Clinch River that encompassed a range of mussel abundance and health, including within a documented zone of mussel decline, to provide insight into the potential role of contaminants in the decline of mussels in the mainstem Clinch River (8 sites) and its tributaries (4 sites). We quantified and related inorganic and organic contaminant concentrations in mussels to their associated habitat compartments (bed sediment, particulate sediment, pore water, and surface water) and found that metals in resident mussels were, for the most part, unrelated to the spatial pattern of variation of metals in all four habitat compartments. However, concentrations of organic contaminants, particularly the polycyclic aromatic hydrocarbons (PAHs) in resident mussels were strongly related to PAH concentrations in surface water, pore water, particulate and bed sediment and the spatial pattern in mussel densities within the river and in zone of mussel decline. This study revealed that PAHs are an important source of contamination in the Clinch River. Accordingly, future conservation and management efforts would benefit by identifying, and ideally mitigating, the sources of PAHs to the river.

Introduction

Native freshwater mussels (Order Unionoida) are one of the most sensitive and rapidly declining faunal groups in the North America. Approximately half of the worldwide unionid diversity exists in North America, but about 70% of the nearly 300 freshwater mussel species are considered vulnerable to extinction or already extinct (Bogan 1993; Williams et al. 1993). These declines have been attributed to numerous factors associated with pollution, water quality degradation, and habitat destruction and alteration (Strayer et al. 2004; Cope et al. 2008; Downing et al. 2010), including the rapid expansion of energy development and other extractive land-uses (Patnode et al. 2015). Indeed, several recent laboratory studies have demonstrated that early life stages of mussels have a high sensitivity to some chemicals related to coal mining and natural gas extraction sites (Wang et al. 2007; Wang et al. 2010; Wang et al. 2013). In field-based studies, inferring specific causes of mussel declines is often difficult because of potential confounding factors and the unique life history of mussels, which makes them differentially susceptible to environmental stressors at several distinct life stages. Nonetheless, chronic, low-level stressors are presumed (Strayer et al. 2004; Cope et al. 2008). Because mussels provide important ecosystem services within freshwater systems (e.g., stabilizing the lotic substrates and sequestering suspended particles and nutrients by filtering stream water (Strayer et al. 1994; Vaughn and Hakenkamp 2001), it is important to identify and quantify the threats to mussels in order to conserve and restore their biodiversity and functional roles.

The Clinch River watershed of the upper Tennessee River basin of Virginia and Tennessee supports one of North America's greatest concentrations of freshwater biodiversity (Neves et al. 1997). This watershed supports 46 extant mussel species, 20 of which are federally

endangered, in addition to 5 federally threatened or endangered fishes (USFWS 2004; Jones et al. 2014). Despite the global biological significance of the Clinch River, mussel populations are declining in some segments, both in species richness and abundance, such that an 88-km reach of the river is considered a mussel "zone of decline" (ZOD; Figure 1). In this river reach from the confluence of the Clinch River with Dumps Creek near the town of Carbo, Virginia (USA; river km 431) to Clinchport, Virginia (USA; river km 343), mussel densities have decreased more than 70% from 1979 to 2004 (Jones et al. 2014). However, both upstream and downstream of the ZOD, stable or even increasing populations of mussels continue to persist (Jones et al. 2014).

The decline in Clinch River mussel populations is a relatively recent event which has occurred mostly after major state and federal environmental laws (e.g., Clean Water Act, Safe Water Drinking Act) were passed in the early 1970s. There are many known historic and present-day stressors to the river, but none have been definitively identified as the cause of the long-term gradual decline in mussel populations. The tributary watersheds of the Clinch River are mixed forests with agriculture, mining, and urban development (Chapter 1, Locke et al. 2006). Specifically, the ZOD has been impacted by wastewater effluents; chemical spills; and runoff and sediment from agriculture, deforested lands, mining influences and urbanization (Ahlstedt 1984; Dennis 1987; Jones et al. 2001; Zimmerman 2003; Ahlstedt et al. 2005; Zipper et al. 2014). In a recent series of journal articles investigating the potential factors contributing to the mussel declines (summarized by Zipper et al. 2014), alterations in physical habitat quality were discounted as a major causative influence. Instead, major ions and water and sediment contaminants were presumed as the primary concerns for mussels, as the authors hypothesized that the most likely explanation for the mussel declines was the sustained (chronic) exposure to non-point pollution and potentially mixtures of toxic chemicals (Jones et al. 2014; Zipper et al.

2014). Given the imperiled status and continued decline of remnant mussel populations in the ZOD, there is a critical and urgent need to thoroughly investigate and understand the effects of chemical these chemical exposures. Furthermore, the strong spatial gradients in mussel health and abundance within this section of the Clinch River provide a unique opportunity to investigate possible mechanisms to explain why mussel health is better in some areas than others (e.g., mussels in the ZOD may have higher body burdens of contaminants).

The aim of this study was to evaluate the exposure of adult resident mussels to a suite of inorganic and organic contaminant stressors throughout a region of the Clinch River that encompassed a range of mussel abundance and health, including within the documented ZOD, to provide insight into the potential role of contaminants in the decline of mussels in the Clinch River and its tributaries. To accomplish this aim, we quantified and related contaminant concentrations in mussels to their associated habitat compartments (bed sediment, particulate sediment, pore water, and surface water).

Methods

Study Area

Within the Clinch River basin, we selected 12 sites encompassing a range of impact on mussel populations (Jones et al. 2014). Eight sites were on the mainstem of the Clinch River and four sites were in tributaries to the Clinch River. Sites located upstream of the ZOD included the mainstem sites of Artrip (ART) and Cleveland (CLE) and one tributary site, Indian Creek (IC). Within the ZOD, there were four mainstem sites; Carterton (CAR), Simones (SIM), Pendleton (PEN), and Clinchport (CHP) and two tributary sites, Dumps Creek (DC) and Guest River (GR). Downstream of the ZOD, there were two mainstem sites, Horton Ford (HF) and Wallen Bend

(WB) and one tributary site, Copper Creek (CC) (Figure 1). Indian Creek, DC, and GR enter the Clinch River from the northwest and drain the Appalachian Plateau physiographic region while CC joins the river from the southwest and drains the Ridge and Valley physiographic region. These tributary sites represent diverse watersheds with varied land uses. Historically, the upper GR has not supported freshwater mussel populations (Johnson et al. 2014), but the watershed has substantial agricultural, mining, and urban development disturbances, in addition to a human health fish consumption advisory due to PCB contamination (Virginia Department of Health 2014). Currently, DC does not support mussel populations and has substantial current and historical coal mining-related disturbances. Both IC and CC support mussel populations, and CC is the second largest sub-watershed to the Clinch River and receives agricultural and rural inputs.

Flows in the sampled section of the Clinch River are variable, but exhibit a strong seasonal pattern of highest flows (20-40 m^3s^{-1}) in the winter and spring (peaking in March), with declining flows over summer and fall and minimal in September, when the long-term (1920-2014) monthly average is ~ 6 m^3s^{-1} [United States Geological Survey (USGS) gage #03524000] deployed at Cleveland, Virginia (http://waterdata.usgs.gov/va/nwis/uv/?site_no=03524000). Due to its long-term record, we used flow data from this gage to provide an indication of how flows during sampling events compared to typical conditions.

Sample Collection

Pore water: We collected pore water from all 12 sites (8 mainstem and 4 tributary sites) during June 5-6, 2013, and October 8-10, 2013. We used a PPX60 PushPoint Sampler (M. H. E. Products, East Tawas, MI, USA) to collect ~5 L of pore water for analyses of metals, organics, and total anions and cations (Ca, Cl, K, Mg, Na, SO_4), total suspended solids, total dissolved solids, alkalinity, total nitrogen, ammonia nitrogen, nitrate, nitrite, and phosphorous from a depth

of 10 cm below the sediment-water interface. Within a site, we changed the location of the sampler placement between each 1 L of water collected. Pore water was pumped through the stainless steel sampler into a 1-L Nalgene™ bottle through clean tubing (Tygon™) that was connected to a peristaltic pump powered by a cordless drill. The first 500 mL of pore water collected was discarded because it contained minor particulates due to sampler placement in the sediment. The pore water samples were preserved with ultrapure (Ultrex™) nitric acid and stored on ice until returning to the laboratory, where the samples were stored refrigerated at 4-10°C until analyses.

Sediment: We collected one composite bed sediment sample at each site on August 21-22, 2012, using a stainless steel scoop rinsed with site water prior to use. Each sample consisted of 3 to 5 scoops from depositional areas within the site, totaling approximately 200 mL. We only collected sediment from the surface layer (top 5 cm) and stored the samples on ice until returning to the laboratory where the samples were stored frozen at -20°C until analyses.

We constructed sediment traps to collect particulate sediment. Traps were 30.5-cm lengths of 7.6-cm diameter PVC pipe with a removable bottom made from the bottom half of a 1-L clear plastic drink bottle. We fastened the removable bottom and sheet of plastic mesh (1-cm mesh size) to exclude large debris to the pipe with stainless steel hose clamps. Traps were designed with an optimal aspect ratio for collecting sediments in lotic conditions (Hargrave and Burns 1979). Two traps were deployed at each site by fastening the traps to a cinder block anchored to the river bottom by a 30.5-cm length of rebar on August 26-28, 2013. We recovered the sediment traps at all sites, except for WB on January 31-February 1, 2014, providing a time-integrated particulate sediment sample. We placed samples in 1-L amber glass jars, transported them on ice to the laboratory, and stored them in an incubator held at 4°C for one week.

Following the holding period, we homogenized the sediment samples, passed them through a 2-mm sieve to remove debris and large particles, and stored the samples frozen at -20°C until analyses.

Surface water (metals): From August 2012 through December 2013, personnel from The Nature Conservancy, Tennessee Department of Environment & Conservation, and Virginia Department of Game & Inland Fisheries (VDGIF) conducted integrated width water sampling approximately quarterly, resulting in five sampling events at eight sites on the mainstem Clinch River: Quarter (Q)1: August 21-23, 2012; Q2: November 15-16, 2012; Q3: April 3-4, 2013; Q4: July 18-19, 2013; Q5: September 18-20, 2013) and three sampling events at the four tributary sites (Q2: October 24-25, 2012; Q3: April 9-10, 2013; Q4: August 5-6, 2013). Surface water samples were analyzed for total and dissolved metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Zn), major ions (Ca, Mg, Na, K, SO_4^{2-}, Cl, and bicarbonate and carbonate alkalinity), total suspended solids, total dissolved solids, and nutrients (total nitrogen, ammonia nitrogen, nitrate + nitrite, and phosphorous).

At least 3 L of water was collected at each site during each sampling event and the samples were fully composited from the width-integrated collections. These water samples were subdivided as needed for parameter measurements and preserved by acidification to a pH of 2 with nitric acid for metals analyses and sulfuric acid for nutrients analyses. The samples were stored and shipped on ice to the U. S. Environmental Protection Agency (EPA) Region 3 Laboratory in Fort Meade, MD, USA, within 72 hours of collection.

Surface water (organics): We deployed passive sampling devices (PSDs) at each of the 12 sites during six separate deployment periods over the course of the study. Passive sampling devices were deployed for approximately one month during each deployment period, thereby providing a

time-integrated water sample for organics analyses. Deployment periods were summer 2012: July 18 – August 21; fall 2012: September 27 – October 24; spring 2013: May 7 – June 5; summer 2013: July 30 – August 26; fall 2013: October 8 – November 5; summer 2014: June 2 – July 8. Two types of PSDs were used, a universal PSD (uPSD) described by O'Neal (2014) and a polyethylene PSD (PE PSD) described by Luellen and Shea (2002), Hewitt et al. (2006), and Cope et al. (2011). Briefly, the uPSD is a stainless steel cartridge constructed from a 10-µm inlet filter (Alltech Associates, Deerfield, IL, USA), with half the volume packed with 200 mg Oasis® HLB sorbent with a particle size of 60 µm and specific surface area of 810 m^2/g (Waters Corporation, Milford, MA, USA). The PE PSD is made from a 25.4-cm X 7.62-cm sheet of polyethylene. Both PSDs were placed inside a plastic mesh cage (5 mm openings) that allowed water to flow past while protecting the PSDs from debris. Upon retrieval, the PSDs were wrapped in baked aluminum foil and placed inside a food-grade sealable plastic bag. The PSDs were stored on ice in the field and frozen at -20°C in the laboratory until analyses.

Mussels: We collected adult resident mussels from the 10 sites with extant mussel populations during October 24-25, 2012 and November 5-7, 2013 (there are no resident mussel populations at GR or DC). At the mainstem sites, we collected 9 *Actinonaias pectorosa* per site per year, and in the tributaries (IC and CC), we collected 9-25 *Villosa iris* per site per year. Mussels were transported on ice to the laboratory where we transferred them to a -20°C freezer for storage. Prior to analyses, mussels were thawed, measured, and weighed and were then homogenized and equally divided to create three composite tissue samples per site. The composite tissue samples were stored frozen (-20°C) until analyses.

Pore water Toxicity Tests

We conducted standard water-only acute (96-h) toxicity tests with juvenile mussels with a portion of the pore water collected from each site in June and October 2013. We began the tests on June 10, 2013, using *Villosa iris* and on October 14, 2013, using *Lampsilis fasciola*. All juveniles were propagated via host-fish infection, using standard propagation and culture methods (Barnhart 2006). The *V. iris* juveniles were obtained from Freshwater Mollusk Conservation Center at Virginia Tech in Blacksburg, VA, and were ~6 weeks old, and the *L. fasciola* juveniles were obtained from VDGIF Aquatic Wildlife Conservation Center in Marion, VA, and were ~8 weeks old at test initiation.

Seven mussels were placed in each of three replicates per treatment, with 10 organisms per replicate in laboratory water controls. The experiment was a static-renewal test and controls were reconstituted hard water (ASTM 2007) to closely approximate water quality conditions in the Clinch River; a 90 – 100% water renewal performed at 48 h. Quality assurance and control were ensured by conducting all tests according to the *Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels* (ASTM 2013). On arrival, mussels were acclimated from their culture water to ASTM reconstituted hard water by placing them in a 50:50 solution of culture/reconstituted water for 2 h, then further diluting the culture water to a 25:75 ratio with reconstituted water, and held for an additional 2 h before being placed in 100% reconstituted water (ASTM 2007; 2013). Mussels were held in ASTM water at 20°C until being distributed into the pore water treatments from each site to start the test. Tests were conducted in light- and temperature-controlled environmental chambers (Precision Model 818, Thermo Fisher Scientific, Marietta, Ohio, USA), held at 20°C and LD 16:8. Water quality was sampled at 48 hours; mean conditions among all treatments in June were 155 mg $CaCO_3$/L alkalinity, 182 mg $CaCO_3$/L hardness, 419 µS/cm conductivity, 8.28 pH, and 8.14 mg/L dissolved oxygen. Individual

treatment means ranged 104 – 254 mg $CaCO_3$/L alkalinity, 112 – 272 mg $CaCO_3$/L hardness, 267 – 591 µS/cm conductivity, 8.15 – 8.47 pH, and 7.88 – 8.33 mg/L dissolved oxygen. Mean conditions among all treatments in October were 219 mg $CaCO_3$/L alkalinity, 195 mg $CaCO_3$/L hardness, 448 µS/cm conductivity, 8.47 pH, and 8.68 mg/L dissolved oxygen. Individual treatment means ranged 148 – 316 mg $CaCO_3$/L alkalinity, 144 – 310 mg $CaCO_3$/L hardness, 328 – 734 µS/cm conductivity, 8.38 – 8.59 pH, and 8.58 – 8.75 mg/L dissolved oxygen. At the end of each 96-h exposure, survival of juvenile mussels was assessed by viewing them under a stereomicroscope; juveniles that exhibited foot movement outside of the shell, foot movement inside the shell, or a detectable heartbeat within a five-minute observation period were considered alive (ASTM 2013).

Laboratory Analyses and Quality Control:

Metals: Metal analyses (Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Ni, Pb, Sb, Se, Si, Sr, V, and Zn) were conducted at RTI International (Research Triangle Park, NC, USA) according to standard methods and approved protocols. Briefly, a 45-mL aliquot from each pore water sample was taken and processed using a modified version of US EPA Method 200.7 to account for the sample size and a 50 mL final volume. For sample digestions, 1.0 mL HNO_3 and 0.5 mL HCl were added to the extraction tubes and heated at 95°C in a SCP Science DigiPrep block digestion unit with watch glasses for 2 h. The samples were then cooled and brought to a final volume of 50 mL using deionized water and vortex-mixed. For sediment and mussels, lyophilized samples were thawed and mixed prior to obtaining a subsample for extraction. A nominal 1.0-g aliquot was used from each of the samples and processed using a modified version of US EPA Method 3050B to account for the sample size and 50 mL final volume. We added 3.0 mL HNO_3 and 1.0 mL HCl to the extraction tubes, heated at 95°C in a

SCP Science DigiPrep block digestion unit, and refluxed for 1 h. We cooled the samples and added 0.5 mL of H_2O_2 to the sample before heating at 95°C, loosely capped, to allow reflux for 1 h. We cooled the samples again, brought them to a final volume of 50 mL using deionized water, and vortex-mixed. We placed the samples in a centrifuge for 20 minutes at 2000 RPM prior to taking an aliquot for measurement.

With the exception of mercury in sediment and mussel tissue, all sample analyses were performed on a Thermo X-Series II ICP-MS or a Thermo iCAP6500 ICP-OES, depending on the concentration of the analyte present in each sample. Each of these instruments was equipped with an autosampler enclosed in a HEPA filtered exhaust box to ensure no adventitious contamination from external sources. These instruments were fully validated according to 21 CFR Part 11, Electronic Records; Electronic Signatures. A rigorous quality assurance protocol was followed during the analysis of each batch of samples and included reagent blanks and reagent blank spikes, duplicate samples, matrix spikes, and standard reference materials. For pore water, relative percent difference (RPD) of duplicates averaged 3.5% (range 0 – 17%), recovery of matrix spikes averaged 94% (range 83 – 108%), reagent blanks were uncontaminated, and average recovery from reagent blanks spikes was 96% (range 91 – 117%). For sediment and mussel tissue, average recovery from standard reference material was 97% (range 57 – 136%), RPD of duplicates averaged 8% (range 0 – 40%), recovery of matrix spikes averaged 99% (range 7 – 212%), reagent blanks were uncontaminated, and average recovery from reagent blanks spikes was 101% (range 96 – 114%).

Mercury concentrations in sediment and mussel tissue was performed using combustion-amalgamation-cold vapor atomic absorption spectrophotometry (Direct Mercury Analyzer 80, Milestone, Monroe, CT, USA) according to U.S. EPA Method 7473. Prior to analysis, the

samples were lyophilized, manually homogenized, and a nominal 0.100-g aliquot of each sample was weighed directly into a nickel boat and placed in the instrument autosampler tray for analysis. Average recovery from standard reference material was 100% (range 94 – 109%), RPD of duplicates averaged 24% (range 2 – 63%), and recovery of matrix spikes averaged 98% (range 72 – 103%).

Metal analyses in surface water was performed by the EPA Region 3 Laboratory, Fort Meade, MD, using standard protocols for determination of surface water quality. US EPA Method 200.7/200.8 was used for all metals except Hg, which used US EPA Method 245.1.

Probable effect concentrations (PEC) were used to characterize sediment toxicity for nine of the 22 metals analyzed (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn; Persaud et al. 1993; MacDonald et al. 2000). Individual PEC quotients (PECQ) were calculated for each metal in each sediment sample as a measure of toxicity by dividing the dry-weight concentration of the metal by its respective PEC, and then deriving the average PECQ for each site. An average PECQ of > 1.0 is considered toxic to aquatic life; however, these sediment quality guidelines were derived from datasets that do not include toxicity to unionoid mussels, and recent research by others has found sediments with values < 0.4 to be toxic to mussels, indicating that current PECs may not be protective of mussels (Wang et al. 2013). To characterize water toxicity, the concentrations of metals analyzed were compared to U.S. EPA's National Recommended Water Quality Criteria for Aquatic Life (accessed 12/07/15, http://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table).

Organics: Organic contaminants analyses were conducted at the North Carolina State University Chemical Exposure Assessment Laboratory in Raleigh, NC, USA, according to standard

methods and approved protocols. Prior to extraction, excess water was removed from uPSDs by centrifugation. All samples were spiked with surrogate internal standards, extracted with organic solvent, and extracts cleaned up and fractionated as described previously for uPSDs (O'Neal 2014), PE PSDs (Hewitt et al. 2006; Cope et al. 2011), mussels (Thorsen et al. 2004), and sediment (Hewitt et al. 2006; Cope et al. 2011). Sample extracts were analyzed for current use pesticides (CUPs, n = 47), polychlorinated biphenyls (PCBs, n = 21), organochlorine pesticides (OCPs, n = 28), polycyclic aromatic hydrocarbons (PAHs, n = 42), and endocrine active chemicals (EACs) using an Agilent 6890 GC connected to an Agilent 5973 mass selective detector operated in selected ion monitoring (SIM) mode. Details of the instrumental analyses have been described previously for CUPs (Hewitt et al. 2006; Cope et al. 2011; O'Neal 2014), OCPs and PCBs (Hewitt et al. 2006; Cope et al. 2011), PAHs (Luellen and Shea 2002; Thorsen et al. 2004; Cope et al. 2011), and EACs (Hirons 2009).

All of the quality control data were within acceptable ranges and validated data quality. Surrogate and matrix spike recoveries for OCPs, PCBs, and CUPs for pore water, mussels, sediment and PSDs were all between 85% and 105%. Because few CUPs, PCBs or OCPs were detected in the samples and those detected were almost always at trace concentrations, these analyte results were not included in further data analysis. Duplicate analysis of matrix spikes and field-collected samples had RPD always below 13% and nearly all below 8%. Surrogate and matrix spike recoveries for the EACs ranged from 91-101% and the RPDs for duplicates were less than 10%. All procedural and field (trip) blanks contained non-detectable target OCPs, PCBs, CUPs, and EACs.

PAHs were detected in all samples and often at high concentrations, so greater detail on quality control results is provided for PAH analysis. Surrogate recoveries for sediment PAH

analysis ranged from 52-102%, with the lower recoveries (52-89%) for the more volatile naphthalene–d8 and acenaphthene–d10 and higher recoveries (59-102%) for the less volatile chrysene-d12 and perylene-d12. Duplicate analysis yielded RPD below 10% for all analytes. Matrix spikes yielded recoveries between 78 and 104%, with RPDs below 10%. Surrogate recoveries for pore water PAH analysis ranged from 72-111%, with the lower recoveries (72-78%) for the more volatile naphthalene–d8 and higher recoveries (88-111%) for the less volatile chrysene-d12 and perylene-d12. Duplicate analysis yielded RPD below 12% for all analytes. Matrix spikes yielded recoveries between 76 and 101%, with RPDs below 10%. Surrogate recoveries for PSD PAH analysis ranged from 62% - 103% with little difference among the four surrogates indicating equal recovery of all PAHs from the PSD samples. Duplicate analysis yielded RPD below 10% for all analytes. Matrix spikes yielded recoveries between 73 and 104%, with RPDs below 10%. Surrogate recoveries for mussel PAH analysis ranged from 71% - 104% with little difference among the four surrogates indicating equal recovery of all PAHs from the mussel samples. Duplicate analysis yielded RPD below 10% for all analytes. Matrix spikes yielded recoveries between 79 and 103%, with RPDs below 10%.

The chemistry data were not corrected for surrogate recoveries because any corrections would have been small and not altered their interpretation. Procedural blanks contained very small amounts of phenanthrene (~ 1 ng in both) and C1-naphthalenes (3 ng and 0 ng). These amounts were well below the concentrations measured in the samples so no blank subtraction was performed.

It should be noted that the fall 2012 PSD sampling event resulted in very high blank contamination for the trip (field) blank and this was mostly due to pyrogenic PAH. PAH results were corrected for this blank contamination, but it is quite likely that the fall 2012 PSD results

are biased high for the pyrogenic PAH due to this unknown source of PAH in the trip blank. This blank contamination problem did not occur with other matrices or sampling times, although smaller amounts of some PAHs were detected in other trip blanks at higher concentrations than commonly observed. In these cases, blank subtraction was not performed because the blanks were far below the measured concentrations in the field samples.

Toxicity of PAH concentrations was evaluated using the most up-to-date EPA methods for evaluating toxicity in sediments, based on a mechanistic approach that incorporates both the additivity of PAH toxicity and the reduction in PAH bioavailability caused by total organic carbon (TOC) in the sediment (USEPA 2003). Raw individual PAH concentrations in each sediment sample were normalized to the TOC fraction and then divided by the respective acute or chronic EPA Potency Divisors (available for 34 PAHs; USEPA 2003) to calculate the equilibrium-partitioning sediment benchmark toxic unit (ESBTU). The individual PAH ESBTUs were totaled; the resulting sum ESBTU (∑ESBTU, hereafter simply referred to as TU) is a measure of Total PAH toxicity. Values ≥ 1.0 indicate the possibility of harm to aquatic life.

Statistical Analyses:

We performed cluster analyses (separately for metals and PAHs) to determine whether groups of sites had similar contaminant signatures and whether any contaminants were similar (i.e., co-varied together) among sites for pore water, sediment, surface water (PAHs only), and mussel tissue concentrations. Specifically, we conducted hierarchical agglomerative cluster analyses using the average group linkage method and Euclidean distances in Statistical Analysis Software (SAS, version 9.3, Cary, NC, USA). We excluded analytes that were below detection limits (BDL) among all sites for a given analysis. However, for analytes that were BDL for some, but not all sites, we substituted half the detection limit for their concentrations. Cluster

analyses were not performed on metal concentrations of discrete water samples because only 8 (Al, Cr, Fe, K, Mg, Mn, Ni, and Zn) of 15 metals were consistently above detection limits in surface water samples. Due to the relatively large variation of concentrations among analytes measured, it was necessary to standardize data (within a constituent) by subtracting the mean (of each metal) and dividing by the standard deviation, to ensure that analytes with high concentrations were not skewing the results (e.g., Fe in pore water ranged from 63 to 15,300 ng/mL while Mo was orders of magnitude less and ranged from 0.1 to 2.0 ng/mL). To determine the optimal number of clusters (groups) in each analysis, we assessed multiple diagnostics, including the pseudo F and pseudo t^2 statistics (generated by SAS) and the relationship between the percent of total variation explained versus the number of clusters (e.g., this would range between 1 and 12 potential clusters because there were 12 sites). These diagnostics generally provided a consensus on the optimal number of clusters because the local maxima of pseudo F often coincided with the point (number of clusters) where pseudo t^2 sharply increased at the next cluster fusion and beyond, at which the amount of variability (information) explained sharply declined, as the number of clusters decreased (i.e., the inflection point). We used PC-ORD (version 6.16, MjM Software Design, Gleneden Beach, OR, USA) to generate two-way dendrograms for each cluster analysis.

We performed correlation analyses to determine the strength of association between all possible pairs of contaminants in mussel tissues (separately and by year for metals and PAHs). For these and all subsequent correlation analyses, to ensure sufficient sample sizes, we only included contaminants that were above detection limits at more than half the sample sites for a given analysis. Furthermore, because a large majority of the data used in our correlation analyses violated the assumption of bivariate normality (required by Pearson correlation), we

instead used Spearman correlation for all analyses, which provides slightly more conservative inferences, but is appropriate for non-normal data. Therefore, we report Spearman's rho correlation coefficients (r_s), which indicate the magnitude and direction of monotonicity between the two variables of interest. For example, positive values of r_s above 0.6 would indicate a strong positive (monotonic) relationship between the concentrations of two given contaminants (e.g., Al and Pb) in mussel tissues in a particular year. We conducted correlation analyses in SAS and used an alpha value of 0.05 to evaluate whether correlation coefficients significantly differed from 0.

We also conducted correlation analyses to determine whether mussel tissue concentrations of analytes were related to those of their immediate environment (i.e., metal and PAH concentrations measured in the sediment, pore water, or surface water at the same sites where mussels were collected). In addition to conducting mussel tissue by sediment correlations on a year-specific basis, we also correlated analyte concentrations of 2013 resident mussels to sediment concentrations in 2012 because we assumed that bed chemistry (measured in 2012) would be relatively stable between years. Correlations of metal tissue with surface water concentrations were conducted by sampling quarter because, with the exception of Mg, metal concentrations were not significantly correlated among water sampling events Q2, Q3, and Q4 (quarters when metals were measured at all 12 sites including the tributaries). Likewise, correlations of PAH tissue concentrations with surface water were performed by deployment period, namely to determine if tissue concentrations were more tightly coupled with recent PAH exposure (i.e., water concentrations of PAHs measured during deployment periods closer in time to the collection date of resident mussels).

Obtaining pore water samples is more time consuming and costly than surface water samples. Therefore, we assessed the feasibility of predicting pore water concentrations of contaminants (metals and PAHs) from surface water measurements by correlating the concentrations of the same contaminants between these two compartments. For these analyses, we used the surface water samples taken closest in time (Q4 and Q5 for metals, spring 2013 and fall 2013 for PAHs) to the pore water samples (June 2013 and October 2013). Only those contaminants that were above detection limits in more than half the samples (surface water or pore water) were included in analyses. We also explored whether there was a relationship between bed and particulate sediment concentrations by correlating the concentrations of given analytes between sediment sources (bed 2012 vs. particulate 2013).

We performed non-parametric multiple comparisons to test for differences in mussel tissue concentrations of given contaminants (metals and PAHs) among sites. A non-parametric approach was necessary due to the non-normality of concentration data, as discussed above. Specifically, we used Dunn's test to conduct all possible pairwise comparisons of ranked concentrations of given contaminants among sites and based our inferences on Bonferroni-adjusted p-values (Zar 1999). These analyses were carried out in JMP Pro (version 12.0.1, SAS Institute, Cary, NC, USA) using an overall (experiment-wise) alpha of 0.05.

To determine if there was an association between mussel density in the Clinch River and body burdens of metals and PAHs, we correlated available mussel density data with measured tissue concentrations of resident mussels. Mussel densities, based on quantitative quadrat sampling (number per m^2), were available for all eight of the mainstem sites from which we collected adult *A. pectorosa* (Krstolic et al. 2013; Jones et al. 2014; J. Jones personal communication). Over the past 10 years (2005-2014), there have been two to three mussel

surveys conducted at the mainstem sites, with the exceptions of CAR, CHP, and HF (1 survey only) and WB (sampled every year). Although the densities reported from these prior and ongoing studies were generally for all mussel species combined, Jones et al. (2014) noted that *A. pectorosa* was common and the dominant species at Virginia sites within the Clinch River. Therefore, we assumed total mussel densities reflected the spatial trend in abundance of our target species *A. pectorosa*. We calculated the mean mussel density at each site (across years) and correlated this with site-specific tissue concentrations of resident mussels within each year (2012 and 2013). As above, we used Spearman correlation and only included in analyses those metals and PAHs that were above detection limits at more than half the sites.

Results

Pore water Toxicity Tests

Pore water collected from the study sites was not acutely toxic to juvenile *V. iris* or *L. fasciola.* In June 2013, we observed 100% survival of *V. iris* in all replicates from the control and site treatments, with the exception of two replicates from WB which had 71 and 86% survival. In October, we likewise observed high survival with 100% survival of *L. fasciola* in all replicates from the control and site treatments, with the exception of 71 or 86% survival in one replicate from CAR, CHP, HF, GR, and CC and two replicates from WB which had 71 and 86% survival each.

Metals

Pore water: Pore water concentrations of metals were much greater in October than in June. River flows were lower during the October (4.7 m^3s^{-1}) sampling event compared to June (11.2 m^3s^{-1}). Of the 252 samples analyzed (21 metals at 12 sites) in each month, 38% were BDL in June compared to only 12% in October; some metals such as Be, Cr, K, and Se were only

detected in the October samples. Even for those metals detected at all sites in both months, concentrations were 2- to 9-fold higher (Ba, Co, Fe, Mn, Ni, Pb, Se, Sr, V; Al 60-fold higher) and exhibited more spatial variability in October than June. For these reasons, only the results of the October samples are presented in-depth herein.

In general, metal concentrations in October pore water samples were higher within the ZOD (e.g., Co and Mn, Figure 2) than outside this zone. However, numerous metals including Al (Figure 2) as well as Be, Cr, Cu, Pb, Se, Zn, and V exhibited low concentrations at GR relative to other sites within the ZOD. Strontium displayed a unique spatial trend with peaks at the DC and GR tributaries (> 375 ng/mL) and low concentrations (< 66 ng/mL) at CAR and CC (Figure 2).

These strong spatial trends in pore water metal concentrations were also evident in the cluster analysis. Three distinct clusters were present and represented the: (1) most upstream and downstream sites including IC, ART, CLE, CHP, CC, HF, WB; (2) mid-river sites within the ZOD including DC, CAR, SIM, PEN; and (3) GR. Overall, the sites comprising cluster 1 had lower metal concentrations than cluster 2 (Figure 3). Cluster 3 was strongly differentiated from cluster 2 by the unique metal signature of GR; that is, higher concentrations of Ba, Sr, Co, Mn, and Mg, but lower concentrations of Al, V, Be, Zn, Cu, Pb, Sb, Cr, and Hg (Figure 3).

Only 11 (Al, As, Cd, Cr, Cu, Fe, Pb, Hg, Ni, Se, Zn) of the 22 metals analyzed in pore water have Aquatic Life Ambient Water Quality Criteria developed by the U. S. EPA. Of these metals, most concentrations were below the criteria, except for several samples of Al and Fe. In the June 2013 pore water samples, CLE (96 µg/L) was above the Al chronic criteria value of 87 µg/L, but for the October 2013 samples, all sites except GR (317 µg/L) were 1.4 to 6.7 times higher than the acute criteria of 750 µg/L. For Fe, in the June 2013 samples, CHP approached

the 1,000 µg/L chronic criteria at 946 µg/L, but WB greatly exceeded it with a concentration of 7,856 µg/L. In addition, all of the October 2013 samples exceed the criteria with concentrations ranging from 1,460 (ART) to 15,300 (DC).

Concentrations of metals in resident mussel tissues were not related to the spatial pattern of variability of metals in pore water, as indicated by the general lack of significant correlations between pore water and mussel tissue concentrations (Table 1). In general, r_s values among metals were below 0.4 with a few exceptions (Figure 4). Resident mussels exhibited a positive relationship with Sr in both June (r_s=0.64, p=0.05) and October (r_s = 0.56, p = 0.09). Furthermore, Ni in resident mussels was negatively related to June pore water (r_s = -0.59, p = 0.07), but positively in October (r_s = 0.68, p = 0.03); Mn was positively related to October pore water (r_s = 0.76, p = 0.01) and Al negatively related to June pore water (r_s = -0.66, p = 0.04).

Sediment: Metal concentrations in the sediment were generally higher in bed (2012) than particulate (2013) samples. Of the 17 metals that were consistently detected, all but one (Cu) exhibited higher concentrations in the bed than particulate sediment. On average, metal concentrations were 50% greater in bed than particulate sediment and this difference ranged from 10% to 92% across 16 metals, excluding Si, which was unique in that it was an order of magnitude higher in the bed (mean = 1,871 ng/mL) versus particulate sediment (mean = 227 ng/mL). All Cd samples were BDL, Sb was only detected in bed samples; Hg, Mo, and Se were detected in only several samples (8-21%) between years).

No strong spatial trends were evident in sediment concentrations throughout the sampling area. Rather, metal concentrations in both bed and particulate sediments varied somewhat randomly among sampling sites ordered by river location (upstream to downstream) (Figure 5 A and B). Furthermore, there appeared to be few metals that were similar among sites in either bed

or particulate sediments, as indicated by the high degree of chaining (i.e., lack of well-defined clusters) in the dendrograms for bed sediment metals (Figure 6) and sites for particulate sediment (Figure 7). In addition, the number of optimal clusters (6 in bed and 8 in particulates) was more than half the maximum number of potential clusters (12 if each site was its own group). Collectively, these results suggest that many sites exhibited unique sediment metal signatures that were not shared by other sites.

Mean metal PECQs for bed sediment for each site ranged from 0.10 to 0.25; sites with the highest values included ART (0.25), HF (0.24), CC (0.21), and SIM (0.20). Individual PECQs for As, Cr, Cu, Fe, Mn, Ni, Pb, and Zn among sites ranged from 0.03 to 0.83, with a mean value of 0.17 among all metals and sites (Table 2). Average individual PECQs among sites for Cu, Pb, and Zn were each < 0.1. PECQs for Cd were not calculated because concentrations were below the detection limit (0.25 µg/g sediment) for all samples. Mn had the highest individual metal PECQ in every sediment sample compared to other metals, averaging 0.43 (range 0.20 – 0.83), with Mn concentrations ranging from 219 to 908 µg/g (Figure 5A). Highest Mn PECQs in the bed sediment were at ART (0.83), CC (0.68) and SIM/HF (both 0.53). The individual PECQ average for Fe and Ni (both 0.22) were also relatively high compared to other metals.

Mean metal PECQs for particulate sediment ranged from 0.10 to 0.17; sites with the highest values included the DC (0.17), CC (0.16), and GR (0.15) tributaries. Individual PECQs for As, Cr, Cu, Fe, Mn, Ni, Pb, and Zn among sites ranged from 0.03 to 0.57, with a mean value of 0.13 (Table 3). Average individual PECQs among sites for As, Cr, Cu, Pb, and Zn were each < 0.1. PECQs for Cd were not calculated because concentrations were below the detection limit (0.25 µg/g sediment) for all sites. Mn had the highest PECQ compared to other metals,

averaging 0.34 (range 0.18 – 0.57) among sites where Mn concentrations ranged 197 – 630 µg/g sediment (Figure 5B). The individual PECQ average for Fe (0.20) was also relatively high compared to other metals.

Tissue concentrations of metals in mussels showed little relation with metal concentrations in the surrounding sediment (Table 4). Most r_s values (77%) for all tissue by sediment correlations were below 0.3 (Figure 4). However, there were a few exceptions, including a marginally significant positive relationship between Zn and particulate sediment (r_s = 0.62, p = 0.07). In addition, a significant negative relationship between K and bed sediment (r_s = -0.71, p = 0.02), and a marginally significant negative relationship between Sb and particulate sediment (r_s = -0.65, p = 0.06).

There was little association between the concentrations of metals in the bed versus particulate sediment. Strontium was the only metal with a significant (positive) relationship between these environmental compartments, although marginally significant relationships were present for K and V (Table 5).

Surface water: We focused on the 8 (Al, Cr, Fe, K, Mg, Mn, Ni, and Zn) of 15 metals which were consistently above detection limits in surface water samples. A salient trend in metal concentrations in surface waters was the strong peak during Q4 of 2013 in which Al, Cr, Fe, Mn, and Ni just downstream of GR, from SIM to CHP were elevated and then attenuated further downstream from CC to WB (Figure 8). The 2013 Q4 sampling event was somewhat unique as it occurred shortly after (< 1 week) a high-flow event that followed a period of otherwise low flows during the preceding 1.5 months (Figure 9). Flows during the other four sampling events occurred under more typical flow conditions; that is, either relatively high spring flows or lower flows in late summer and fall (Figure 9). During sampling events other than Q4 2013, metals

exhibited much lower concentrations and little spatial trend, except for the high values of Mn at GR and IC and low Ni (BDL) at CC during Q3 (Figure 8). Magnesium showed a very similar spatial pattern among sites during Q2, Q3, and Q4, with peaks at GR and CC (Figure 8). Potassium exhibited peaks at DC and GR, but not at the other tributaries sampled (IC and CC) (Figure 8).

As with pore water, only 11 (Al, As, Cd, Cr, Cu, Fe, Pb, Hg, Ni, Se, Zn) of the 15 metals analyzed in surface water have Aquatic Life Ambient Water Quality Criteria developed by the U.S. EPA. Of these metals, most concentrations were below the criteria except for several samples of Al, Fe, and Zn. For Al, the chronic criterion (87 µg/L) was exceeded in 35% of the samples and the acute (750 µg/L) was exceeded in 2 samples in the high-flow event of Q4. Also in Q4, the Fe chronic criterion (1,000 µg/L) was exceeded in at 2 sites (Dungannon upstream of SIM and CHP). In addition, the criterion for Zn (120 µg/L) was exceeded at 3 sites (CLE, CAR, and PEN) in Q1.

While the majority (86%) of correlations between metal concentrations in mussel tissues and those in surface water were not significant, the water column was the environmental compartment in which we observed the strongest positive correlations with mussel tissue concentrations (Figure 4). Specifically, mussel tissue concentrations exhibited significant or marginally significant positive correlations with surface water Ni during three sampling events (Q2, Q4, and Q5), Mn during two sampling events (Q2 and Q4), and Al (Q5), Cr (Q5), and Zn (Q4) during single sampling events (Table 4).

Metal concentrations in surface water did not generally track pore water concentrations among sampling sites. The only significant correlation between these two variables was for Mg, which showed a significant positive relationship for the June 2013 pore water sample, but not the

October 2013 sample (Table 7). Nickel exhibited a marginally significant positive relationship in October 2013, but no relationship (r_s = -0.08) for the June 2013 sample (Table 7).

Resident Mussels: The tissue concentrations of most metals (12 of 21), including Al, Co, Cr, Cu, Fe, K, Mo, Ni, Pb, Sb, Si, and V, varied randomly among sites with little spatial trend (Figure 10). However, some metals including Ba, Hg, Mg, Mn, Se, Sr, and Zn exhibited a distinct spatial trend whereby tissue concentrations were elevated at three of the mid-river sites (SIM, PEN, and CHP) in the ZOD, but concentrations at CAR (within the ZOD) were relatively low, and at WB (outside the ZOD), relatively high (Figure 10). Overall, mussel tissue concentrations were higher and more variable among sites in 2012 than 2013 with the exception of V (Table 8; Figure 10); nonetheless, most metals (83%) exhibited a positive correlation (with 9 of 18 being significant) between years, suggesting a consistent spatial trend in metal accumulation across years (Figure 10). Two exceptions were As and Cd, which displayed a spatial trend similar to Mn (described above) in 2012 but varied little in 2013 (Figure 10). Finally, some sets of metals: (1) Ba, Mg, Mn, Se, Sr, Zn, Se, and Sr and (2) Al and Si appeared similar (co-varied) between years as they clustered together each year (Figures 11 and 12) and were positively and significantly correlated with one another (Table 9).

Mussel densities at the sites showed little association with metal tissue concentrations. There were no significant correlations between mussel density and the site-specific tissue concentrations of any metals in either year, with the exception of Cr in 2013 (Table 10). However, the correlation with Cr is likely spurious due to the limited range of Cr values measured in 2013 (1.86-3.37 ng/L) compared to 2012 (2.3-22.4 ng/L), when no significant relationship was observed (r_s = -0.17, p = 0.69).

Organics

Pore water: In the June 2013 pore water samples, PCBs were detected at 5 sites, with the highest concentrations of total PCBs (sum of all PCBs analyzed) occurring at ART (19.3 ng/L) and the remaining concentrations at CLE, CAR, CHP, and CC ranging between 1.2-2.3 ng/L. No PCBs were detected in the October 2013 samples. In June 2013, the only OCPs detected were lindane at two sites (10.9 ng/L at ART and 8.2 ng/L at CLE), but no OCPs were detected in October samples. For CUPs, three herbicides and one herbicide metabolite were prominently detected. Atrazine was measured at 7 sites, and the atrazine metabolite, atrazine-desethyl at 2 sites. Metolachlor was measured at 4 sites in the June 2013 sample, where PEN had the highest concentrations of each: 12.2, 4.4, and 7.1 ng/L, respectively. In October 2013, atrazine was again measured at 7 sites, with the highest concentration of 2.3 ng/L found at ART. In addition, tebuthiuron was measured at all sites except CAR with concentrations ranging from 5.26 ng/L (CC) to 22.5 ng/L (GR).

PAHs were measured at all sites in June 2013, and the concentrations of total PAHs (sum of the 42 PAHs analyzed) ranged from 0.37 ng/L at HF to 15.9 ng/L at CLE. There was little difference among sites in total PAH concentrations for either the June or October samples, with the exception of a large peak in PAHs at DC (114 ng/L) in October (Figure 13), which is also evident in the cluster analysis for that sample (Figure 14).

There was little association between tissue concentrations in resident mussels and corresponding pore water concentrations of individual PAHs, as indicated by the general lack of significant correlations between pore water and PAH tissue concentrations (Table 11). In general, r_s values among PAHs were below 0.4 (Figure 4) with a few exceptions, namely the significant positive relationship between P1 in PAHs and mussel tissue in June ($r_s = 0.82$), but

not October (r_s = 0.20) (Table 11). Furthermore, total PAH concentrations in mussels did not show a strong association with either June (r_s = 0.26) or October (r_s = 0.39) pore water samples.

Sediment: In the bed sediment collected in 2012, no CUPs were detected and concentrations of PCBs and OCPs were below 1 ng/g. In the particulate sediment collected in 2013-2014, OCPs were not detected, except at GR, where a low concentration (2.5 ng/g) of trans-nonachlor was measured. No PCBs or CUPs were detected in particulate sediments at any sites.

PAHs were widespread and measured in every bed and particulate sediment sample analyzed. Concentrations of total PAHs in the bed sediment were above 1,000 ng/g at all sites except IC and CC. Total PAHs in the bed sediment ranged from a low of 250 ng/g (dry weight) at CC to over 7,000 ng/g at GR and SIM (Figure 15a). A spatial trend was present whereby total PAHs in the bed sediment were higher at sites within the ZOD (Figure 15A). In general, the PAH concentrations were lower in the bed sediment than the particulate sediment collected in 2013-2014 which ranged from 1,300 ng/g (dry weight) at IC to 54,000 ng/g at CAR with substantial differences at some sites (Figure 15B). For example, the total PAH at CAR was 4,600 ng/g in bed sediment, but 54,000 ng/g in particulate sediment. This change was dominated by pyrogenic PAHs in the particulate sediment. Similar to the bed sediment samples, a spatial trend was evident whereby total PAH concentrations in the particulate sediment were considerably higher at sites within the ZOD (Figure 15b).

Cluster analyses supported the strong spatial trends of PAHs in both the bed and particulate sediment. Three distinct clusters were evident in the 2012 bed sediment samples and represented: (1) mid-river sites within the ZOD (CAR, GR, SIM, PEN, and CHP), in addition to ART; (2) sites both upstream (IC, CLE) and downstream (CC, HF, WB) of the ZOD; and (3) DC. Overall, the sites comprising clusters 1 and 3 had higher PAH concentrations than cluster 2

(Figure 16). Particulate sediment samples (2014) also grouped into three distinct clusters which represented the following groupings: (1) ART, CC, CLE, IC, SIM, PEN, and HF, four of which were outside the ZOD; (2) three sites within the ZOD including CHP, DC, and GR; and (3) CAR. Clusters 2 and 3 generally had higher PAH concentrations than cluster 1 (Figure 17).

Mussel tissue concentrations of most PAHs exhibited a strong positive correlation with those in the surrounding sediment (Table 12). Most r_s values were above 0.4, specifically: 76% in 2012, and 85% in 2013 for the tissue by bed sediment correlations, and 58% for the tissue by particulate sediment correlations in 2013 (Figure 4). Moreover, nearly half of all correlation analyses between tissue and bed sediment PAH concentrations were significant.

In the 2012 bed sediment, no sites exceeded the EPA benchmark for PAHs, although DC had a TU of 0.4 and all sites had elevated PAHs compared to background concentrations. For the 2014 particulate sediment, TU values ranged from 0.004 to 0.37 for acute toxicity and 0.018 to 1.51 for chronic toxicity. Only CAR exceeded a TU of 1.0, but several sites were clearly higher than others, including SIM, PEN, HF, GR, and especially DC and CHP, where TU values for both were above 0.40. Most particulate sediments were dominated by petrogenic PAHs (i.e., fossil fuel-sourced including most of the PAH from unburned coal), accounting for 65 – 85% of Total PAH compared to the pyrogenic portion (i.e., sourced from combustion/burning of fossil fuels). Petrogenic and pyrogenic PAHs were more evenly represented in Clinchport sediment (55 and 45%, respectively). Contrary to most other treatments, pyrogenic PAHs were the dominant source in Carterton and Copper Creek sediments, accounting for 71 and 64% (respectively) of all PAHs in those sediments, and petrogenic PAHs contributing ≤ 36%.

Many PAHs exhibited a strong positive correlation between the bed (2012) and particulate (2013) sediment. Of the 26 PAHs that exhibited a monotonic trend between these compartments, for which correlation was appropriate, all but 2 (C4 and RET) displayed a significant positive relationship (Table 13, Figure 18A). More than one-third of the PAHs displayed a non-linear (polynomial) and non-monotonic relationship (Figure 18B) that was not appropriate for Spearman correlation or for general predictive purposes.

Surface water (PSDs): Generally, concentrations of PCBs were not detected in surface waters using either the uPSD or PE PSDs, indicating very low concentrations (< 1 ng/L) of highly weathered PCBs. The exception in 2013 was the detection of several PCB congeners at most sites in the PE PSD during the spring and summer (highest total PCB concentration at GR was 7.9 ng/L), with a single PCB detected in the fall deployment, but none in the uPSDs. In summer 2014, low concentrations of PCBs were detected at several sites in the PE PSDs, with the highest concentration of total PCBs (7.3 ng/L) again occurring at GR.

There were no OCPs detected in any of the uPSDs in summer or fall 2012. However, low concentrations of chlordanes were detected in the PE PSDs that were deployed in fall 2012. Concentrations were highest (~30 ng/L) at the GR site. In 2013, concentrations of OCPs were somewhat higher compared to 2012. In spring and summer 2013, chlordanes were detected in PE PSDs at all sites and concentrations were highest at GR, exceeding 50 ng/L in spring. This site also had the highest concentrations of heptachlor epoxide (6.8 ng/L) and dieldrin (18 ng/L in the fall) and highest concentration of 2,4'-DDD (9.9 ng/L in spring). In fall 2013, chlordanes were lower (e.g., ~16 ng/L in GR) as were the other OCPs detected. In summer 2014, OCPs were similar to 2013 with chlordanes detected at all sites except CHP and concentrations at GR were

again the highest (~60 ng/L) and the highest concentrations of heptachlor epoxide (5.2 ng/L) and 4,4'-DDE (6.2 ng/L).

In 2012, the only CUPs detected in PSDs were atrazine (8.1 ng/L) and atrazine-desethyl metabolite (3.4 ng/L) at CLE during the summer deployment. In 2013, the herbicide tebuthiuron was detected in all uPSD samples in the summer deployment (concentrations ranging from 2.5 ng/L at WB to 7.3 ng/L at SIM), but no detections were observed in spring or fall deployment periods. The only other detections were 2-7 ng/L atrazine in spring and summer, and 6.5 ng/L metolachlor at CHP in spring 2013. In 2014, atrazine (15 -20 ng/L) and metolachlor (7-35 ng/L at all mainstem sites except PEN which was 94 ng/L) were detected at more sites and at higher concentrations than previous years.

PAHs were detected in PSDs at all sites and in all sampling periods. In 2012, during the summer deployment, total PAH concentrations ranged from near blank levels at DC and CC to about 80-100 ng/L for most other sites and up to ~1,000 ng/L at GR (Figure 19). However, even at GR, the TU value for protection of aquatic life was not exceeded for PAHs, as the maximum was below 0.32 for chronic exposure to PAHs, and all other sites were well below 0.10 TU. At most sites, PAH concentrations were slightly lower in the fall 2012 PE PSDs than in the summer 2012 uPSDs. During both deployments, GR had, by far, the highest total PAH concentrations (800 ng/L) followed by sites just downstream of GR. There was a shift in the relative abundance of PAH from summer to fall, whereas the summer was dominated by petrogenic PAHs and the fall by either an equal mix of petrogenic and pyrogenic PAH or predominantly pyrogenic PAHs. The only sites that retained the dominant petrogenic PAH signature during both fall and summer 2012 were CC and DC. It should be noted that the fall sampling event resulted in very high blank contamination for the trip (field) blank and this was mostly due to pyrogenic PAHs. Our results

were corrected for this blank contamination, but it is quite likely that the fall 2012 results are biased high for the pyrogenic PAH due to this unknown source of PAH in the trip blank. Even with the possibility of PAH contamination in the fall 2012 sampling, the TU hazard quotients for fall were less than those in the summer.

As with 2012, in 2013 all PSDs at all sites and deployments periods had elevated concentrations of PAHs. The PAH concentrations in the PE PSDs were generally consistent between the spring, summer, and fall 2013 sampling. Total PAH concentrations were generally higher in 2013 compared to 2012 (Figure 19), with GR exceeding 5,200 ng/L in the fall. In general, PAH concentrations based on the PE PSD data were somewhat higher in fall compared to spring and summer 2013, and for most sites the TU values were higher in summer compared to fall or spring. This is due to a higher relative abundance of the more toxic high molecular weight PAH in the summer compared to the fall and spring. Once again, GR was highest and the TU value exceeded 1.0. The TU value approached 1.0 for a number of other sites as well (IC, ART, CLE, DC, SIM, CHP, and HF).

Total PAH concentrations in the PSDs for 2014 were similar to 2013 for most sites (Figure 19) with GR exceeding 6,000 ng/L. Nonetheless, chronic TU values were higher and exceeded 1.0 for CLE, PEN, SIM, and GR and were above 0.5 for IC, ART, CAR, DC, and HF. These higher TU values reflect a greater abundance of the higher molecular weight PAHs compared to prior years.

Similar to the relation with sediment, mussel tissue concentrations of many PAHs exhibited a strong positive relationship with their surrounding surface water concentrations, as measured by the PSDs (Table 14, Figure 4). In 2012, PAH concentrations from the fall PSD deployment period, closest in time to the date mussels were collected, were strongly correlated

with mussel PAH concentrations as r_s values ranged from 0.60 to 0.93 (with one exception), and 16 of 18 correlations were significant (Table 14). In 2013, when only half as many individual PAHs were detected compared to 2012, PAH concentrations from the summer deployment had the most significant positive relationships with mussel tissue concentrations (nearly half of the correlations were significant) (Table 14).

The concentrations of PAHs in surface waters, as measured in PSDs, did not generally track pore water concentrations across sampling sites. The only significant correlations between these two variables were for F and N4 with the June 2013 pore water sample (Table 15). Surface water and pore water concentrations of N1 exhibited a significant positive relationship for the October 2013 pore water sample, but not the June 2013 sample (r_s = -0.16) (Table 15).

The EACs BisphenolA and nonylphenol were detected at most sites in 2012, 2013, and 2014, with concentrations in the low ng/L range or lower. The GR site had the highest concentrations of EACs; bisphenol A ranged from 1-3 ng/L and nonylphenol ranged from 3-8 ng/L. No hormones were positively identified in the samples; only 17-α ethinylestradiol (EE2), an oral contraceptive drug was tentatively identified in all samples at low ng/L concentrations, but the mass spectra were not definitively confirmed as EE2.

Mussel Tissue: In 2012, concentrations of total PCBs were generally less than 1 ng/g (dry weight) at all sites, indicating very low accumulation of PCBs in mussels. However, the highest concentrations (0.8-1.0 ng/g) were found at the upstream sites (ART, CLE, and CAR), while all other sites had concentrations <0.3 ng/g. The PCBs detected were primarily those in the 5-6 Cl range (101, 118, 153, 105, 138) and individual PCB congener concentrations were below 1 ng/g. No PCBs were detected in any samples in 2013.

In 2013, only a few samples (at CC) contained detectable quantities of any OCPs, and these consisted of very low concentrations of 4,4'-DDE and 2,4'-DDD, with all detections well below 1 ng/g. No OCPs were detected in 2013. In addition, no CUPs were detected in any mussel samples.

Unlike other organic compounds, PAHs were prevalent in mussel tissues. In general, PAH concentrations in resident mussels were higher in 2013 (ranging from 194 ng/g at CC to 1,073 ng/g at SIM) than in 2012 (ranging from 38 ng/g at CC to 978 ng/g at SIM) (Figure 20). Interestingly, there were fewer individual PAHs detected in 2013 (13 PAHs) than 2012 (21 PAHs) (Table 16, Figures 21 and 22) and fewer significant differences in PAHs among individual sites in 2013 (7 PAHs) than in 2012 (10 PAHs) (Tables 17 and 18). Of the 10 PAHs that were detected in mussels both years, 7 were significantly correlated ($p < 0.05$) between years ($r_s = 0.66$-0.81) as well as total PAH concentrations (sum of 42 measured) ($r_s = 0.89$), providing support for a consistent spatial trend in PAH accumulation between years in mussels.

Strong spatial trends were present in PAH concentrations in mussel tissues that coincided with the ZOD. The highest tissue concentrations of total PAHs in both years occurred at sites within the ZOD (CAR to CHP) compared to sites upstream (IC, ART, CLE) and downstream (CC, HF, WB) of this zone (Figure 20). In 2012, cluster analyses revealed that PEN and SIM, which are just downstream of GR, had high relative concentrations and a unique PAH signature from other sites (Figure 21). The 2013 cluster analysis revealed relatively high concentrations of individual PAHs at CHP and a spatial pattern that differed from the remaining sites, but also high relative concentrations in several PAHs at CAR, SIM, and PEN (Figure 22). The spatial trends and PAH clustering were supported by the results of the paired PAH correlations, which

indicated most PAHs within the same cluster (group) were highly correlated with one another (Table 16).

In contrast to the relation observed with metals, mussel densities exhibited a strong negative relationship with tissue PAH concentrations. With one exception (AN), all individual PAHs in 2012 showed a moderate to strong negative relationship with site-specific mussel density as r_s values ranged from -0.45 to -0.93 and most correlations (14 of 18) were at least marginally significant (p-values < 0.1) (Table 19). Similarly, in 2013, moderate to strong negative relationships were observed for all PAHs (r_s values ranged from -0.41 to -0.71), but only one such relationship (N1) was significant. Mussel densities were also strongly correlated with total PAH (sum of 42 measured) tissue concentrations in both years (Table 19). The trend displayed in Figure 23, whereby the highest mussel densities clearly occurred at the lowest total PAH concentrations, followed by declining densities thereafter as tissue concentrations increased, was representative of the pattern displayed by the individual PAHs that exhibited a negative relationship with mussel density.

Discussion

Mussel populations in portions of the Clinch River have declined substantially in recent decades, but the causes of this decline remains unknown. In this study, we evaluated the exposure of adult resident mussels to inorganic and organic contaminants in multiple habitat compartments (surface water, sediment pore water, bed sediment, particulate sediment) and examined their relation to regions of the Clinch River that differ in mussel population density, both in areas of stability (outside the ZOD) or in areas of declining in abundance (within the ZOD). While metal contaminants are present in the Clinch River and have received the most attention (Zipper et al. 2014), spatial trends in metal concentrations in mussels and in the

environmental compartments were not consistent with mussel population metrics and were highly variable among sites and compartments. Conversely, spatial trends in mussel populations closely aligned with trends in certain organic contaminants, specifically PAHs. Our study represents one of the most comprehensive investigations of organic contaminants in the Clinch River watershed to date (see also: Hampson et al. 2000, Johnson et al. 2014). We found legacy and current use pesticides, as well as PCBs, to be of little concern to aquatic life in the river. However, with coal mining a major landscape activity in parts of the watershed (see Chapter 1 in this report, Figure 5), it is not surprising that PAHs were prevalent at all sites with higher concentrations in all compartments found at sites within the ZOD. Results from our study supported previous research (e.g., Jones et al. 2014) that suggested chronic stressors and potentially synergistic effects of contaminant mixtures are a principle concern for mussel populations.

In general, spatial trends in metal concentrations in mussels and the environmental compartments were unrelated and were highly variable among sites and compartments. Some metals peaked at sites within the ZOD in some compartments (see appendices A-C), but overall, there were little relation between metal concentrations in the environment and mussel tissue or between site-specific mussel densities and mussel tissue concentrations. However, there were observed trends towards higher metal concentrations at some sites in the ZOD, especially downstream from GR, compared to the more upstream and downstream sites; this trend was especially evident for mining-associated metals (e.g., Al, Fe, Mg, Mn, Se, and Zn) in the October 2013 pore water samples and mussel tissue concentrations (particularly in 2012 when concentrations were higher) (Figure 11; appendices A and C; also note Cd and Sr in mussels have a similar trend). Concentrations in some of these metals (Al, Fe, and Zn [surface water

only]) also exceeded U.S. EPA's water quality criteria for protection of aquatic life in pore water and surface water at some sampling events. Moreover, Taskinen et al. (2011) found that elevated concentrations of Al (0.25-1.0 mg/L) and Fe (0.5-2.0 mg/L) negatively affected survival of glochidia and juveniles of *Margaritifera margaritifera* in an acute 72 h exposure. Neither bed nor particulate sediment concentrations of these same mining-associated metals exhibited this trend. The highest mean metal PECQs for metals (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in bed sediment was 0.25 (ART) and 0.24 (HF), which are both outside of the ZOD and are likely below levels which we would expect to elicit negative effects, even based on the updated studies that identify some sediments with PECQs greater than 0.2 as toxic to freshwater mussels (Wang et al. 2013). Therefore, metal contaminants alone cannot explain the decline of mussels in the ZOD in the Clinch River.

PAHs are the organic contaminants of most concern in the Clinch River based on our findings. The highest PAHs in both the mussels and the environmental compartments were at sites within the ZOD. Additionally, in contrast to metals, there were clear relationships between mussel tissue concentrations and concentrations in the more time-integrative samples of sediment and surface water (via measurement with PSDs). This is also in contrast to a recent study in the Clinch River by Johnson et al. (2014) who found the lack of a spatial trend in PAHs in bed sediment. We calculated acute and chronic TUs as an indicator of toxicity in the sediment and surface water where a value of 1.0 or greater indicates possibility of harm to aquatic life. The highest chronic TU in the bed sediment was 0.4 at DC, but in particulate sediment, CAR exceeded the accepted toxicity threshold at 1.51, and other sites within the ZOD had relatively high values (0.44 at DC and 0.48 at CHP; range in ZOD was 0.16 to 1.51). Due to a PAH signature with more PAHs of high molecular weight, surface water TUs exceeded 1.0 at GR and

approached 1.0 at all other sites (except CAR, PEN, CC, and WB for some deployment periods in 2013) and exceeded 1.0 for CLE, GR, SIM and PEN in 2014. Furthermore, in contrast to metals, we found a strong negative relationship between PAH concentrations in mussel tissue and mussel densities, providing additional evidence that PAHs are a major contaminant of concern and are a potential causal factor in the decline of mussels within the ZOD. Additional studies may be warranted to examine the mechanistic relationships of PAH exposure and mussel health.

Based on the locations of the greatest measured PAH concentrations, there is not a clear or dominant attributable landscape source of PAHs in the Clinch River watershed. However, the tributaries in the mining-intensive parts of the watershed (see Chapter 1 in this report) appear to be an important contributing source, especially at the GR site, which was found to have PAH concentrations greater than most other sites in the Clinch River (especially in the surface water). The DC site clearly had a different PAH signature than the other sites (especially in the October 2013 pore water and bed sediment). However, the highest PAH concentrations were found in the particulate sediment samples at CAR which is upstream from the GR site. The CAR site is unusual in that it had a high abundance of pyrogenic (combustion-related) PAHs compared to the other sites that mostly had a stronger petrogenic (oil-related) PAH signature. However, CAR is situated just downstream of a coal-fired power plant along the Clinch River at Carbo (Virginia, USA). While this explanation is an oversimplification of PAH source, especially because coal has a PAH signature that is a mix of petrogenic and pyrogenic sources, it is clear that the PAHs found in these sediments are consistent with a predominant influence of coal-related PAHs. Equally important to note is that the non-mining impacted tributaries (IC and CC) and the downstream sites (HF and WB) had lower PAH concentrations than those in the ZOD.

Compared to previous studies on the Clinch River (e.g., Johnson et al. 2014), this study highlighted the dynamic nature of this watershed. With the advantage of sampling the Clinch River and its tributaries over two years, we were able to resolve annual and seasonal differences and how they affected the periodicity of contaminant exposure to mussels. For example, concentrations of metals in mussels were generally higher and more variable in 2012 than 2013, when a series of high flow events occurred in late spring through the summer (April-Sept) resulting in higher median spring-summer flows (14.2 m^3s^{-1}) compared to 2012 (9.5 m^3s^{-1}). In contrast, PAH concentrations were generally higher in 2013 than 2012, but the PAH signatures differed between years as fewer individual PAHs were present in 2013. Despite the yearly differences in magnitude, most metals and PAHs (those detected in both years) in mussel tissues were positively correlated between years, indicating an overall continuity in the spatial pattern of contaminant accumulation and putative sources. Surprisingly, these observations indicate that the body burdens of metals and PAHs in mussels are of recent rather than long-term (multiple-year), integrative contaminant exposure. This hypothesis is further supported by the observations that contaminant concentrations in mussels were generally more highly correlated with concentrations in the environmental compartments closer to the time of mussel collection and resident mussels were more highly correlated with juvenile mussels (deployed in the same year residents were collected, see Chapter 3 of this report) than between years (i.e., 2012 vs. 2013 correlations of contaminants with resident mussels).

While our study generally supports the prevailing hypothesis on mussel declines in the Clinch River, it provides new information on the potential role of PAHs as contaminants at a local scale, which should extend to management and conservation efforts for aquatic ecosystems elsewhere. Johnson et al. (2014) postulated that mussel populations in the ZOD are continually

exposed to flow draining tributaries of mined watersheds (e.g., DC and GR) of degraded water quality in the Appalachian Plateau, but tributaries flowing through the Valley and Ridge physiographic provinces (e.g., IC and CC), unaffected by mining, allow for stable and even expanding mussel populations upstream and downstream of the ZOD. While our study did not detect strong relations to conductivity and major ions between surface water and mussels, which have previously been found to be higher in areas with low-quality mussel assemblages (Johnson et al. 2014; Price et al. 2014), we found higher concentrations of certain metals associated with mining in the ZOD. Moreover, our study revealed that PAHs are an additional, and potentially very important source of contamination, due to the high concentrations and toxicities found within the ZOD and the negative relationships with mussel densities in the Clinch River. Accordingly, future conservation and management efforts would benefit by identifying, and ideally mitigating, the sources of PAHs to the Clinch River. Lastly, it is important to note the importance of contaminant mixtures as contributing stressors to mussels in the Clinch River, which have been previously observed for sensitive aquatic species (Gautier et al. 2014; Gillis 2012).

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Tables

Table 1. Spearman's rho correlation coefficients for correlations between site-specific June and October 2013 pore water metal concentrations and 2013 resident mussel metal tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from analyses.

Metal	*June 2013*	*October 2013*
Al	***0.66****	0.10
As	BDL	-0.12
Ba	0.26	0.54
Be	BDL	BDL
Cd	BDL	BDL
Co	0.32	0.48
Cr	BDL	0.19
Cu	-0.34	0.15
Fe	0.04	0.36
Hg	BDL	BDL
K	BDL	0.05
Mg	0.04	0.21
Mn	0.14	***0.76****
Mo	0.10	0.16
Ni	***-0.59***	***0.68****
Pb	-0.25	0.25
Sb	BDL	BDL
Se	BDL	BDL
Si	BDL	BDL
Sr	***0.64****	***0.56***
V	0.07	0.13
Zn	0.26	0.22

Table 2. Probable effect concentration quotients (PECQs) of individual metals in each bed sediment sample for which Probable Effect Concentrations (PECs) are available (Persaud et al. 1993; MacDonald et al. 2000), and the mean metal PECQ for each sediment sample (zone of decline sites shaded grey). Metal concentrations (µg/g sediment) and PECs (mg/kg dry weight sediment) are in equivalent units; PECQ = sediment concentration/PEC.

Metal	PEC	IN	ART	CLE	DC	CAR	GR	SIM	PEN	CHP	CC	HF	WB
As	**33**	0.15	0.17	0.11	0.12	0.10	0.08	0.12	0.13	0.10	0.18	0.27	0.12
Cr	**111**	0.04	0.13	0.09	0.05	0.09	0.04	0.11	0.10	0.09	0.24	0.23	0.13
Cu	**149**	0.03	0.07	0.05	0.05	0.05	0.05	0.07	0.08	0.05	0.04	0.08	0.06
Fe	**40000**	0.18	0.32	0.21	0.22	0.20	0.16	0.23	0.23	0.22	0.24	0.23	0.23
Mn	**1100**	0.20	0.83	0.33	0.38	0.27	0.30	0.45	0.53	0.35	0.68	0.53	0.37
Ni	**48.6**	0.12	0.25	0.18	0.20	0.18	0.20	0.27	0.29	0.19	0.18	0.33	0.25
Pb	**128**	0.04	0.11	0.08	0.05	0.08	0.05	0.07	0.10	0.08	0.07	0.12	0.08
Zn	**459**	0.08	0.12	0.08	0.09	0.07	0.10	0.09	0.12	0.09	0.06	0.12	0.09
Mean Metal		0.10	0.25	0.14	0.14	0.13	0.12	0.18	0.20	0.14	0.21	0.24	0.16

Table 3. Probable effect concentration quotients (PECQs) of individual metals in each particulate sediment sample for which Probable Effect Concentrations (PECs) are available (Persaud et al. 1993; MacDonald et al. 2000), and the mean metal PECQ for each sediment sample (zone of decline sites shaded grey). Metal concentrations (µg/g sediment) and PECs (mg/kg dry weight sediment) are in equivalent units; PECQ = sediment concentration/PEC.

Metal	PEC	IN	ART	CLE	DC	CAR	GR	SIM	PEN	CHP	CC	HF
As	**33**	.11	.06	.06	.11	.06	.06	.06	.06	.07	.10	.08
Cr	**111**	.07	.06	.07	.07	.07	.04	.06	.07	.08	.11	.09
Cu	**149**	.10	.09	.08	.16	.08	.09	.10	.08	.09	.08	.08
Fe	**40000**	.19	.17	.20	.26	.21	.16	.20	.19	.22	.23	.21
Mn	**1100**	.18	.20	.32	.38	.27	.57	.32	.35	.39	.49	.33
Ni	**48.6**	.11	.10	.12	.16	.12	.17	.13	.13	.15	.13	.12
Pb	**128**	.05	.06	.07	.07	.07	.06	.07	.06	.07	.08	.06
Zn	**459**	.06	.07	.07	.13	.07	.09	.07	.06	.07	.06	.06
Mean Metal		.11	.10	.12	.17	.12	.15	.13	.13	.14	.16	.13

Table 4. Spearman's rho correlation coefficients for correlations between site-specific bed (2012) and particulate (2013) sediment and resident mussel metal tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from our analyses.

Metal	2012 Residents *Bed*	2013 Residents *Bed*	2013 Residents *Particulate*
Al	-0.36	0.03	0.13
As	-0.07	0.20	0.08
Ba	0.14	0.42	0.23
Be	BDL	BDL	BDL
Cd	BDL	BDL	BDL
Co	0.05	0.22	0.38
Cr	0.21	0.02	-0.17
Cu	-0.45	-0.09	-0.55
Fe	0.14	-0.22	-0.02
Hg	BDL	BDL	BDL
K	***-0.71****	-0.34	0.07
Mg	-0.04	-0.05	0.30
Mn	0.10	0.11	-0.08
Mo	BDL	BDL	BDL
Ni	0.48	0.36	0.50
Pb	-0.22	-0.12	0.42
Sb	BDL	BDL	***-0.65***
Se	BDL	BDL	BDL
Si	-0.30	0.36	0.37
Sr	0.2	0.30	0.37
V	-0.04	0.07	-0.08
Zn	0.48	0.49	***0.62***

Table 5. Spearman's rho correlation coefficients (r_s) for correlations between the site-specific mean concentrations of metals in bed (2012) vs. particulate sediment (2013). Bold and italics denote marginal significance ($0.05 < p < 0.1$), while bold and italics with an asterisk indicate significance ($p \leq 0.05$).

Metal	r_s
Al	0.14
As	0.44
Ba	0.50
Co	0.11
Cr	0.27
Cu	-0.24
Fe	0.21
K	***0.53***
Mg	0.41
Mn	0.12
Ni	0.12
Pb	0.21
Si	0.41
Sr	***0.64****
V	***0.53***
Zn	0.08

Table 6. Spearman's rho correlation coefficients for correlations between site-specific surface water concentrations of metals (by sampling event) and resident mussel metal tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from our analyses. Sampling events were as follows: Q1=August 21-23, 2012 (mainstem only); Q2= October 24-25, 2012 (tributaries) and November 15-16, 2012 (mainstem); Q3=April 3-4, 2013 (mainstem) and April 9-10, 2013 (tributaries); Q4=July 18-19, 2013 (mainstem) and August 5-6, 2013 (tributaries); Q5=September 18-20, 2013 (mainstem only). Resident mussels were collected during October 24-25 in 2012 and November 5-7 in 2013.

	2012		*2013*		
Metal	Q1	Q2	Q3	Q4	Q5
Al	0.21	-0.50	-0.27	-0.24	***0.76****
Cr	BDL	BDL	BDL	-0.35	***0.63***
Cu	BDL	BDL	BDL	-0.36	BDL
Fe	0.24	BDL	0.12	-0.04	-0.01
K	- 0.50	0.09	-0.03	-0.22	0.04
Mg	0.30	0.20	0.19	-0.12	-0.32
Mn	0.26	***0.59***	0.07	***0.58***	-0.07
Ni	-0.05	***0.73****	0.39	***0.83****	***0.83****
Zn	-0.20	BDL	BDL	***0.81****	0.24

Table 7. Spearman's rho correlation coefficients for correlations between the site-specific concentrations of metals in June 2013 pore water samples (June PW) vs. those measured in surface waters during July 18-19, 2013; correlations of site-specific metal concentrations in October 2013 pore water samples (Oct PW) vs. those measured in surface waters during September 18-20, 2013. BDL = more than half of samples were below detection limit; therefore, the metal was excluded from our analyses. Bold and Italics only = marginal significance ($0.05 < p < 0.10$).

Metal	June PW	Oct PW
Al	0.36	0.52
Cr	BDL	-0.05
Fe	0.23	0.48
K	BDL	0.37
Mg	***0.6****	0.48
Mn	0.25	-0.19
Ni	-0.08	***0.69***
Zn	0.20	0.38

Table 8. Results from Dunn non-parametric multiple comparisons of metal concentrations among sites for resident mussels by year, 2012 and 2013. Within a column, sites with different letters indicate significant differences (i.e., where Bonferonni-adjusted p-values ≤ 0.05). Only those metals with significant pairwise differences are included here.

	2012 residents								*2013 residents*	
Site	As	Ba	Co	Cr	K	Mn	Si	Sr	Site	Sr
IC	ab	ab	ab	ab	a	ab	ab	ab	IC	ab
ART	ab	ab	ab	a	Ab	ab	a	ab	ART	ab
CLE	ab	ab	ab	ab	Ab	ab	ab	ab	CLE	ab
DC	ab	ab	ab	ab	Ab	ab	ab	ab	DC	ab
CAR	ab	ab	ab	ab	Ab	ab	ab	ab	CAR	ab
GR	ab	ab	ab	ab	Ab	ab	ab	ab	GR	ab
SIM	ab	ab	ab	ab	Ab	ab	ab	ab	SIM	a
PEN	a	a	a	ab	Ab	a	ab	a	PEN	ab
CLP	ab	ab	ab	ab	B	ab	ab	ab	CLP	ab
CC	ab	ab	ab	ab	Ab	ab	ab	ab	CC	b
HF	b	b	b	b	Ab	b	b	b	HF	b
WB	ab	ab	ab	ab	ab	ab	ab	ab	WB	ab

Table 9. Matrix reporting Spearman's rho correlation coefficients (r_x) among all possible pairs of site-specific mean tissue concentrations of metals in resident mussels within each year. The top, off-diagonal, portion of the matrix (above the black line) shows r_x values for 2012 correlations and the bottom, off-diagonal, portion shows r_x values for 2013 correlations. Cells with gray-fill and bolded and italicized text indicate r_x values were significantly different than 0.

																				2012	
	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Hg	K	Mg	Mn	Mo	Ni	Pb	Sb	Se	Si	Sr	V	Zn	
Al		0.14	-0.37	-0.30	0.56	***0.70***	***0.71***	0.56	-0.19	***0.70***	-0.27	-0.41	***0.67***	0.05	***0.94***	***0.78***	-0.44	***0.99***	-0.35	***0.98***	-0.43	Al
As	-0.20		0.49	***0.71***	***0.75***	0.42	-0.22	***0.72***	***0.72***	-0.26	0.49	0.45	0.04	0.04	0.22	0.04	0.38	0.13	0.50	0.13	0.42	As
Ba	***-0.72***	0.30		***0.89***	0.27	0.13	-0.61	-0.02	0.47	***-0.68***	***0.94***	***0.98***	***-0.68***	0.52	-0.19	-0.36	***0.94***	-0.36	***0.95***	-0.37	***0.96***	Ba
Cd	0.03	0.58	0.26		0.38	0.04	-0.54	0.22	0.55	-0.50	***0.77***	***0.84***	-0.44	0.31	-0.21	-0.25	***0.81***	-0.31	***0.90***	-0.30	***0.82***	Cd
Co	-0.25	0.53	***0.75***	***0.71***		***0.82***	0.33	***0.83***	0.37	0.16	0.36	0.24	0.30	0.35	***0.65***	0.58	0.07	0.58	0.20	0.50	0.16	Co
Cr	0.03	0.33	0.16	0.30	0.31		0.42	***0.68***	0.22	0.14	0.24	0.08	0.22	0.27	***0.83***	0.61	-0.07	***0.76***	-0.03	***0.64***	0.05	Cr
Cu	***0.68***	-0.12	-0.50	0.02	-0.35	0.31		0.45	-0.50	***0.85***	-0.60	-0.68	***0.68***	-0.08	0.58	***0.76***	***-0.70***	***0.70***	***-0.66***	***0.71***	***-0.73***	Cu
Fe	-0.08	***0.76***	0.01	0.45	0.21	0.20	0.07		0.36	0.28	-0.05	-0.13	0.47	-0.19	0.55	0.54	-0.20	0.58	-0.09	0.55	-0.16	Fe
Hg	-0.38	0.45	0.43	0.56	***0.68***	0.12	***-0.73***	0.18		***-0.64***	0.47	0.44	-0.05	-0.21	-0.13	-0.31	0.44	-0.18	0.39	-0.27	0.41	Hg
K	0.32	-0.22	***-0.75***	-0.22	***-0.71***	-0.18	0.48	0.25	-0.56		***-0.67***	***-0.71***	***0.76***	-0.05	0.49	***0.78***	***-0.72***	***0.66***	-0.60	***0.70***	***-0.73***	K
Mg	-0.62	0.19	***0.95***	0.35	***0.77***	0.19	-0.42	-0.15	0.45	***-0.76***		***0.98***	-0.62	***0.67***	-0.04	-0.30	***0.89***	-0.26	***0.90***	-0.30	***0.95***	Mg
Mn	-0.53	0.27	***0.95***	0.35	***0.83***	0.10	-0.47	-0.09	0.45	***-0.82***	***0.93***		***-0.71***	0.61	-0.20	-0.38	***0.93***	-0.39	***0.95***	-0.42	***0.99***	Mn
Mo	-0.11	0.43	0.28	***0.87***	***0.69***	0.03	-0.38	0.21	***0.81***	-0.34	0.37	0.39		-0.36	0.43	***0.64***	***-0.66***	0.62	-0.62	0.60	***-0.77***	Mo
Ni	-0.26	0.10	***0.71***	0.33	***0.70***	0.31	-0.19	-0.10	0.21	-0.50	***0.67***	***0.81***	0.30		0.24	0.21	0.42	0.07	0.52	0.02	0.58	Ni
Pb	-0.01	0.62	0.45	***0.76***	***0.87***	***0.64***	-0.03	0.37	0.55	-0.42	0.49	0.53	0.61	0.60		***0.70***	-0.30	***0.95***	-0.20	***0.93***	-0.21	Pb
Sb	-0.18	-0.36	0.14	0.12	0.09	-0.25	-0.47	-0.22	0.32	-0.22	0.10	0.21	0.36	0.31	-0.14		-0.55	***0.82***	-0.41	***0.70***	-0.41	Sb
Se	-0.55	0.22	***0.95***	0.39	***0.84***	0.10	-0.45	-0.03	0.45	***-0.72***	***0.94***	***0.98***	0.42	***0.82***	0.55	0.24		-0.47	***0.95***	-0.42	***0.92***	Se
Si	***0.95***	-0.32	***-0.66***	0.07	-0.18	0.07	0.59	-0.26	-0.24	0.20	-0.49	-0.47	-0.01	-0.25	0.03	-0.10	-0.48		-0.37	***0.95***	-0.41	Si
Sr	-0.56	0.32	***0.89***	0.38	***0.85***	0.14	-0.61	0.10	0.60	***-0.68***	***0.82***	***0.92***	0.47	***0.82***	0.61	0.32	***0.94***	-0.52		-0.32	***0.94***	Sr
V	-0.47	0.21	***0.88***	0.48	***0.83***	0.08	-0.45	-0.19	0.55	***-0.84***	***0.93***	***0.95***	0.56	***0.72***	0.52	0.31	***0.93***	-0.35	***0.83***		-0.43	V
Zn	***-0.73***	0.32	***0.99***	0.19	***0.72***	0.19	-0.55	-0.03	0.45	***-0.78***	***0.93***	***0.94***	0.24	***0.68***	0.44	0.10	***0.92***	***-0.67***	***0.88***	***0.85***		Zn
	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Hg	K	Mg	Mn	Mo	Ni	Pb	Sb	Se	Si	Sr	V	Zn	
	2013																					

Table 10. Spearman's rho correlation coefficients from correlations of site-specific mean total mussel densities vs. metal tissue concentrations measured in resident mussels in 2012 and 2013. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from our analyses. Mussel densities were based on quantitative quadrat surveys conducted intermittently over the years 2005-2014 by Krstolic et al. 2013, Jones et al. 2014, and J. Jones (personal communication).

Metal	2012	2013
Al	-0.48	0.38
As	-0.43	-0.17
Ba	-0.24	-0.29
Cd	-0.38	-0.12
Co	-0.36	-0.24
Cr	-0.17	***-0.88****
Cu	0.00	-0.19
Fe	-0.38	-0.50
Hg	0.07	0.00
K	-0.24	-0.31
Mg	-0.33	-0.24
Mn	-0.29	-0.17
Mo	-0.24	BDL
Ni	-0.26	***-0.67***
Pb	-0.36	-0.55
Sb	-0.31	0.10
Se	-0.43	-0.29
Si	-0.38	0.40
Sr	-0.50	-0.33
V	-0.57	0.00
Zn	-0.31	-0.26

Table 11. Spearman's rho correlation coefficients for correlations between site-specific June and October 2013 pore water PAH concentrations and 2013 resident mussel PAH tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$). BDL = more than half of samples were below detection limit; therefore, the PAH was excluded from our analyses.

PAH	*June 2013*	*October 2013*
AN	-0.01	BDL
C	-0.13	BDL
F	0.25	BDL
FL	0.47	BDL
FP1	0.21	BDL
N1	0.27	0.28
N2	0.14	BDL
N3	0.20	BDL
P	BDL	0.47
P1	***0.82****	0.20
PY	0.43	BDL
Sum34	0.26	0.39
Sum42	0.26	0.39

Table 12. Spearman's rho correlation coefficients for correlations between site-specific surface water concentrations of PAHs (by PSD deployment period) and resident mussel PAH tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the PAH was excluded from our analyses. Seasonal deployments of PSDs were as follows: 2012 Summer = July 18-August 21, 2012; 2012 Fall 2012 = September 27-October 24; 2013 Spring 2013 = May 7-June 5; 2013 Summer 2013 = July 30-August 26; 2013 Fall = October 8-November 5. Resident mussels were collected during October 24-25 in 2012 and November 5-7 in 2013.

PAH	2012 Summer	2012 Fall	2013 Spring	2013 Summer	2013 Fall
Ace	0.27	***0.60***	BDL	BDL	BDL
AN	BDL	***0.83****	***0.65***	***0.81****	0.43
BaA	0.24	***0.87****	BDL	BDL	BDL
C	BDL	***0.87****	-0.15	0.21	0.26
D	BDL	***0.71****	BDL	BDL	BDL
F	BDL	***0.84****	0.10	***0.61***	0.31
F1	BDL	***0.64****	BDL	BDL	BDL
F2	BDL	***0.93****	BDL	BDL	BDL
F3	BDL	BDL	BDL	BDL	BDL
FL	0.35	***0.67****	0.13	***0.71****	0.45
FP1	BDL	***0.84****	-0.05	0.10	0.04
N	***0.71****	BDL	BDL	BDL	BDL
N1	***0.80****	BDL	-0.02	0.43	***0.55***
N2	BDL	BDL	BDL	0.21	0.54
N3	BDL	***0.92****	0.53	***0.67****	***0.57***
N4	0.43	***0.83****	BDL	BDL	BDL
P	BDL	***0.77****	0.38	***0.73****	***0.61***
P1	BDL	BDL	0.45	***0.58***	0.41
P2	BDL	***0.82****	BDL	BDL	BDL
PER	BDL	0.28	BDL	BDL	BDL
PY	BDL	***0.79****	-0.25	0.30	0.47
Sum34	***0.69****	***0.81****	-0.03	0.42	0.33
Sum42	***0.56****	***0.81****	-0.03	0.42	0.32

Table 13. Spearman's rho correlation coefficients (r_s) for correlations between mean site-specific concentrations of PAHs in bed (2012) vs. particulate sediment (2013). 'N/A' denotes PAHs that were not analyzed due to a non-linear (polynomial) and non-monotonic relationship between the two variables. Bold and italics denote marginal significance ($0.05 < p < 0.1$), while bold and italics with an asterisk indicate significance ($p \leq 0.05$).

PAH	r_s	PAH	r_s
Ace	N/A	F1	***0.84****
Acy	N/A	F3	***0.82****
AN	N/A	FL	N/A
BaA	N/A	FP1	***0.77****
BaP	N/A	FP2	***0.78****
BbF	N/A	FP3	***0.79****
BeP	***0.75****	IDP	N/A
BgP	***0.87****	N	***0.81****
BkF	N/A	N1	***0.93****
C	N/A	N2	***0.90****
C1	N/A	N3	***0.93****
C2	***0.74****	N4	***0.95****
C3	***0.80****	P	N/A
C4	0.46	P1	***0.77****
COR	N/A	P2	***0.79****
D	***0.77****	P3	***0.79****
D1	***0.79****	P4	***0.78****
D2	***0.80****	PER	N/A
D3	***0.71****	PY	N/A
DBA	***0.85****	RET	0.40
F	***0.66****		

Table 14. Spearman's rho correlation coefficients for correlations between site-specific surface water concentrations of PAHs (by PSD deployment period) and resident mussel PAH tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the PAH was excluded from our analyses. Seasonal deployments of PSDs were as follows: 2012 Summer = July 18-August 21, 2012; 2012 Fall 2012 = September 27-October 24; 2013 Spring 2013 = May 7-June 5; 2013 Summer 2013 = July 30-August 26; 2013 Fall = October 8-November 5. Juvenile mussels were deployed from June 5 to October 25 in 2012 and from July 31 to November 7 in 2013.

PAH	2012 Summer	2012 Fall	2013 Spring	2013 Summer	2013 Fall
Ace	***0.51***	***0.97****	BDL	BDL	BDL
AN	BDL	***0.73****	***0.58***	***0.94****	0.45
BaA	***0.64****	***0.99****	BDL	BDL	BDL
C	BDL	***0.96****	0.05	0.20	0.48
D	BDL	***0.80****	BDL	BDL	BDL
F	BDL	***0.95****	0.10	***0.71****	0.25
F1	BDL	***0.66****	BDL	BDL	BDL
F2	BDL	***0.90****	BDL	BDL	BDL
F3	BDL	BDL	BDL	BDL	BDL
FL	***0.74****	BDL	-0.02	***0.70****	0.29
FP1	BDL	***1.00****	0.29	0.35	0.22
N	***0.97****	BDL	BDL	BDL	BDL
N1	0.38	BDL	0.05	0.30	0.48
N2	BDL	BDL	0.18	0.42	***0.64****
N3	BDL	***0.92****	***0.73****	***0.71****	***0.74****
N4	***0.54***	***0.71****	BDL	BDL	BDL
P	BDL	***0.89****	0.35	0.43	0.17
P1	BDL	BDL	0.51	***0.55***	0.34
P2	BDL	***0.81****	BDL	BDL	BDL
PER	BDL	0.22	BDL	BDL	BDL
PY	BDL	***0.96****	-0.11	0.28	0.39
Sum34	***0.69****	***0.98****	0.48	0.45	***0.55***
Sum42	***0.64****	***0.98****	0.48	0.45	***0.57***

Table 15. Spearman's rho correlation coefficients for correlations between the site-specific concentrations of PAHs in June 2013 pore water samples (June PW) vs. those measured during the spring 2013 PSD deployment (May 7-June 5); correlations of site-specific PAH concentrations in October 2013 pore water samples (Oct PW) vs. those measured in surface waters during the fall 2013 PSD deployment (October 8-November 5). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from our analyses. Bold and Italics only = marginal significance ($0.05 < p < 0.10$).

PAH	June PW	Oct PW
AN	-0.45	BDL
C	0.15	BDL
F	***0.81****	BDL
F1	-0.02	BDL
FL	-0.04	BDL
FP1	0.04	BDL
N1	-0.16	***0.69****
N2	***-0.62***	BDL
N3	0.05	BDL
N4	***0.67****	BDL
P	BDL	0.14
P1	0.21	-0.06
P2	0.02	BDL
PY	-0.07	BDL

Table 16. Matrix reporting Spearman's rho correlation coefficients (r_x) among all possible pairs of site-specific mean tissue concentrations of PAHs in resident mussels within each year. The top, off-diagonal, portion of the matrix (above the black line) shows r_x values for 2012 correlations and the bottom, off-diagonal, portion shows r_x values for 2013 correlations. Cells without values (dashes) represent PAHs for which there were too few samples (i.e., below detection limits at more than half of the sampling sites) to conduct correlation analyses. Cells with gray-fill and bolded and italicized text indicate r_x values were significantly different than 0.

2012

	Ace	AN	BaA	C	C1	D	D2	F	F1	F2	F3	FL	FP1	N	N1	N2	N3	N4	P	P1	P2	PER	PY	
Ace		***0.72***	***0.67***	***0.72***	-0.23	0.28	-0.41	***0.72***	0.50	0.38	0.08	***0.71***	***0.63***	***0.66***	0.24	0.02	***0.70***	0.59	0.38	-	***0.72***	0.27	0.61	**Ace**
AN	-		***0.74***	***0.85***	-0.29	0.41	0.02	***0.99***	***0.81***	***0.75***	0.60	***0.90***	***0.79***	***0.83***	***0.76***	0.42	***0.88***	***0.78***	0.62	-	***0.89***	***0.66***	***0.78***	**AN**
BaA	-	-		***0.81***	0.06	***0.67***	-0.23	***0.80***	***0.71***	0.52	0.20	***0.94***	***0.98***	***0.71***	0.53	0.38	***0.94***	***0.93***	***0.68***	-	***0.89***	***0.67***	***0.96***	**BaA**
C	-	***0.71***	-		-0.29	0.36	-0.05	***0.87***	***0.79***	0.54	0.50	***0.93***	***0.84***	***0.72***	0.60	0.34	***0.85***	***0.89***	0.53	-	***0.93***	***0.76***	***0.85***	**C**
C1	-	-	-	-		0.31	0.36	-0.17	-0.41	0.06	-0.26	-0.17	-0.06	-0.41	-0.47	-0.19	-0.06	-0.06	0.29	-	-0.17	-0.06	-0.17	**C1**
D	-	-	-	-	-		-0.15	0.46	0.46	0.63	0.25	0.52	***0.65***	0.29	0.24	***0.64***	0.61	***0.65***	0.28	-	0.52	0.55	0.60	**D**
D2	-	-	-	-	-	-		0.08	0.02	0.35	0.60	-0.10	-0.22	-0.05	0.14	0.04	-0.13	-0.05	0.32	-	-0.10	0.35	-0.28	**D2**
F	-	***0.97***	-	***0.77***	-	-	-		***0.82***	***0.78***	0.60	***0.93***	***0.83***	***0.84***	***0.74***	0.42	***0.90***	***0.82***	***0.70***	-	***0.90***	***0.71***	***0.81***	**F**
F1	-	0.06	-	-0.06	-	-	-	0.06		***0.71***	***0.69***	***0.83***	***0.77***	***0.88***	***0.85***	***0.73***	***0.73***	***0.77***	0.55	-	***0.75***	***0.77***	***0.81***	**F1**
F2	-	-	-	-	-	-	-	-	-		***0.77***	***0.63***	0.55	0.57	0.55	***0.69***	***0.64***	0.60	0.59	-	0.61	***0.69***	0.49	**F2**
F3	-	-	-	-	-	-	-	-	-	-		0.44	0.29	0.51	***0.66***	0.60	0.36	0.45	0.29	-	0.44	***0.76***	0.29	**F3**
FL	-	***0.79***	-	***0.82***	-	-	-	***0.85***	-0.17	-	-		***0.96***	***0.83***	***0.71***	0.41	***0.98***	***0.95***	***0.69***	-	***0.98***	***0.77***	***0.95***	**FL**
FP1	-	0.29	-	0.12	-	-	-	0.27	0.59	-	-	0.05		***0.75***	***0.64***	0.45	***0.96***	***0.95***	***0.66***	-	***0.93***	***0.72***	***0.99***	**FP1**
N	-	-	-	-	-	-	-	-	-	-	-	-	-		***0.83***	0.39	***0.76***	***0.71***	0.62	-	***0.75***	0.61	***0.76***	**N**
N1	-	0.51	-	0.28	-	-	-	0.59	0.29	-	-	0.42	0.08	-		0.58	0.63	0.59	0.52	-	0.62	***0.64***	***0.68***	**N1**
N2	-	0.18	-	0.35	-	-	-	0.21	0.52	-	-	-0.04	0.53	-	0.35		0.37	0.43	0.24	-	0.33	0.54	0.48	**N2**
N3	-	0.31	-	0.57	-	-	-	0.40	0.41	-	-	0.19	0.36	-	0.26	***0.83***		***0.94***	***0.69***	-	***0.98***	***0.73***	***0.93***	**N3**
N4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0.59	-	***0.95***	***0.88***	***0.92***	**N4**
P	-	***0.67***	-	0.59	-	-	-	***0.81***	0.06	-	-	***0.81***	0.25	-	0.59	0.13	0.33	-		-	0.60	0.51	0.60	**P**
P1	-	***0.68***	-	0.47	-	-	-	***0.75***	0.29	-	-	***0.75***	0.33	-	0.58	0.10	0.31	-	***0.77***		-	-	-	**P1**
P2	-	0.42	-	0.49	-	-	-	0.51	-0.17	-	-	0.34	0.38	-	0.37	0.53	0.55	-	0.53	0.22		***0.78***	***0.90***	**P2**
PER	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		***0.70***	**PER**
PY	-	***0.87***	-	***0.67***	-	-	-	***0.90***	0.29	-	-	***0.79***	0.40	-	***0.75***	0.31	0.31	-	***0.79***	***0.76***	0.42	-		**PY**
	Ace	**AN**	**BaA**	**C**	**C1**	**D**	**D2**	**F**	**F1**	**F2**	**F3**	**FL**	**FP1**	**N**	**N1**	**N2**	**N3**	**N4**	**P**	**P1**	**P2**	**PER**	**PY**	

2013

Table 17. Results from Dunn non-parametric multiple comparisons of PAH concentrations among sites for resident mussels in 2012. Within a column, sites with different letters indicate significant differences (i.e., where Bonferonni-adjusted p-values < 0.05). Only those PAHs with significant pairwise differences are included here.

Site	Ace	BaA	C1	F1	F2	FP1	N4	P	PER	PY	Site
IC	ab	ab	a	ab	ab	ab	ab	ab	ab	ab	IC
ART	ab	ab	b	ab	a	ab	ab	ab	ab	ab	ART
CLE	ab	ab	b	ab	ab	ab	ab	ab	ab	ab	CLE
CAR	a	ab	b	ab	ab	ab	ab	ab	a	ab	DC
SIM	ab	a	b	a	b	a	a	ab	ab	a	CAR
PEN	b	a	b	ab	ab	ab	a	ab	ab	ab	GR
CHP	ab	ab	b	ab	ab	ab	ab	a	ab	ab	SIM
CC	ab	ab	ab	ab	ab	ab	ab	ab	ab	ab	PEN
HF	ab	ab	b	ab	a	ab	ab	ab	ab	ab	CHP
WB	ab	b	b	b	ab	b	b	b	b	b	CC

Table 18. Results from Dunn non-parametric multiple comparisons of PAH concentrations among sites for resident mussels in 2013. Within a column, sites with different letters indicate significant differences (i.e., where Bonferonni-adjusted p-values < 0.05). Only those PAHs with significant pairwise differences are included here.

Site	AN	F	F1	FL	N2	P	PY	Site
IC	ab	ab	a	ab	ab	ab	ab	IC
ART	ab	ab	a	ab	ab	ab	ab	ART
CLE	ab	ab	a	ab	ab	a	ab	CLE
CAR	ab	ab	b	ab	ab	ab	ab	DC
SIM	ab	ab	a	ab	a	ab	ab	CAR
PEN	ab	ab	a	ab	ab	ab	ab	GR
CHP	a	a	a	a	b	b	a	SIM
CC	ab	b	a	b	ab	a	ab	PEN
HF	ab	ab	a	ab	ab	ab	ab	CHP
WB	b	b	a	b	ab	ab	b	CC

Table 19. Spearman's rho correlation coefficients from correlations of site-specific mean total mussel densities vs. PAH tissue concentrations measured in resident mussels in 2012 and 2013. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the PAH was excluded from our analyses. Mussel densities were based on quantitative quadrat surveys conducted intermittently over the years 2005-2014 by Krstolic et al. 2013, Jones et al. 2014, and J. Jones (personal communication).

PAH	2012	2013
Ace	-0.19	BDL
AN	***-0.67***	-0.40
BaA	***-0.62***	BDL
C	-0.45	-0.43
D	***-0.65***	BDL
F	***-0.67***	-0.52
F1	***-0.93****	BDL
F2	***-0.83****	BDL
FL	***-0.64***	-0.40
FP1	***-0.62***	-0.61
N	***-0.74****	BDL
N1	***-0.90****	***-0.71****
N2	BDL	-0.55
N3	-0.60	-0.48
N4	***-0.64***	BDL
P	***-0.67***	-0.57
P1	BDL	-0.60
P2	-0.52	BDL
PER	***-0.83****	BDL
PY	***-0.62***	***-0.69***
Sum42	***-0.64***	***-0.83****

Figure legends

Figure 1. Study area, the Clinch River of Virginia and Tennessee, and sampling sites for resident mussels and environmental concentrations of contaminants. The solid black dots denote the 8 mainstem sampling sites (site names bolded), and white triangles indicate the 4 tributary sites (site names italicized). The 'Zone of Decline', downstream of Cleveland to Clinchport, represents a 68 km stretch of river where mussel populations have declined over the past 30 years (Jones et al. 2014).

Figure 2. Representative spatial trends of pore water metal concentrations. Pore water samples were collected during June 5-6, 2013 (gray diamonds) and October 8-10, 2013 (black squares). Tributary sites are denoted by asterisks. Lines connecting points are for visual presentation and do not reflect connectivity between means.

Figure 3. Two-way dendrogram from cluster analysis of pore water metal concentrations among sites for the October 2013 pore water sampling. Relative metal concentrations are indexed by the color gradient, as darker red cells indicate higher concentrations of a given metal in the pore water at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 12 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains. For example, in this dendrogram, our statistical analyses determined the optimal number of clusters to be 3 (coded by color); by grouping sites into these 3 clusters, ~70% of the original variation (information) among all 12 sites was retained.

Figure 4. Boxplots illustrating the strength of association between resident mussel tissue concentrations of contaminants (metals and PAHs) and their corresponding concentrations in various environmental compartments (pore water, sediment, and surface water). The boxplots show the distribution of Spearman rho correlation coefficients (r_s) for each set of analyses [i.e., all correlations run for each combination of contaminant type (metals or PAHs) by environmental compartment]. For example, the second box plot shows the distribution of r_s values for all 17 correlations run between tissue and pore water concentrations of PAHs across sampling events (June and October 2013). Note for sediment that r_s values are only shown for bed sediment (not particulate).

Figure 5. Representative spatial trends of (A) bed and (B) particulate sediment metal concentrations. Bed sediment samples were collected during August 21-22, 2012 (black diamonds) and particulate sediment samples were collected from August 26-28, 2013 to January

31-February 1, 2014 (gray squares). Tributary sites are denoted by asterisks. Lines connecting points are for visual presentation and do not reflect connectivity between means.

Figure 6. Two-way dendrogram from cluster analysis of bed sediment metal concentrations among sites. Relative metal concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the bed sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 12 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 7. Two-way dendrogram from cluster analysis of particulate sediment metal concentrations among sites. Relative metal concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the particulate sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 12 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 8. Spatial trends of surface water metal concentrations by sampling event for the 8 metals above the detection limit (DL). Dashed line indicates ½ DL. Sampling events were as follows for mainstem: (Q1) August 21-23, 2012; (Q2) November 15-16, 2012; (Q3) April 3-4, 2013; (Q4) July 18-19, 2013; and (Q5) September 18-20, 2013) and tributary sites: (Q2): October 24-25, 2012; (Q3): April 9-10, 2013; (Q4): August 5-6, 2013). Tributary sites are denoted with an asterisk. Lines connecting points are for visual presentation and do not reflect connectivity between means.

Figure 9. Daily mean flows during years 2012 (blue line) and 2013 (red line) near the Cleveland (CLE) sampling site in the Clinch River at USGS gage 03524000 (http://waterdata.usgs.gov/

nwis/uv?site_no=03524000). The dates of surface water sampling events (Q1-Q5) and pore water (PW) sampling (2013 only) are overlain as symbols on the hydrograph. For surface water sampling events, circles represent the mainstem sites and triangles the tributary sites (PW sampling in mainstem and tributary sites occurred closer in time and are therefore represented by the same symbol, squares).

Figure 10. Representative spatial trends of resident mussel metal concentrations. Mussels were collected during October 24-25, 2012 (black circles) and November 5-7, 2013 (gray circles).

Means and standard errors were based upon 3 composite samples (of 9 individuals) from each site. Tributary sites are denoted by asterisks. Lines connecting points are for visual presentation and do not reflect connectivity between means.

Figure 11. Two-way dendrogram from cluster analysis of resident mussel metal concentrations among sites in 2012. Relative metal concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the particulate sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 10 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 12. Two-way dendrogram from cluster analysis of resident mussel metal concentrations among sites in 2013. Relative metal concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the particulate sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 10 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 13. Spatial trends of pore water total PAH concentrations. Pore water samples were collected during June 5-6 (gray diamonds) and October 8-10 (black squares), 2013. Tributary sites are denoted with asterisks. Lines connecting points are for visual presentation and do not reflect connectivity between means.

Figure 14. Two-way dendrogram from cluster analysis of pore water PAH concentrations among sites in October 2013. Relative PAH concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the bed sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 12 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 15. Spatial trends of total PAH concentrations in the (A) bed and (B) particulate sediment. Bed sediment samples (black diamonds) were collected during August 21-22, 2012 and particulate sediment samples (gray squares) were collected from August 26-28, 2013 to January 31-February 1, 2014. Tributary sites are denoted with asterisks. Lines connecting points are for visual presentation and do not reflect connectivity between means.

Figure 16. Two-way dendrogram from cluster analysis of bed sediment PAH concentrations among sites. Relative PAH concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the bed sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 12 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 17. Two-way dendrogram from cluster analysis of particulate sediment PAH concentrations among sites. Relative PAH concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the particulate sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 12 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 18. Representative relationships for PAHs that showed a: (a) polynomial and non-monotonic and (b) strong positive relationship between concentrations measured in the bed (2012) and particulate (2013) sediment. r_s indicates the Spearman's rho correlation coefficient.

Figure 19. Spatial trends total PAH concentrations in surface water during the six (~1 month-long) PSD deployments. Deployment periods were as follows: summer 2012= July 18-August 21; fall 2012=September 27-October 24; spring 2013=May 7-June 5; summer 2013=July 30-August 26; fall 2013=October 8-November 5; summer 2014=June 2-July 8. Tributary sites are denoted by asterisks. Lines connecting points are for visual presentation and do not reflect connectivity between means.

Figure 20. Spatial trends of resident mussel total PAH concentrations. Mussels were collected during October 24-25, 2012 (black circles) and November 5-7, 2013 (gray circles). Means and standard errors were based upon 3 composite samples (of 9 individuals) from each site. Tributary sites are denoted by asterisks. Lines connecting points are for visual presentation and do not reflect connectivity between means.

Figure 21. Two-way dendrogram from cluster analysis of resident mussel PAH concentrations among sites in 2012. Relative PAH concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the particulate sediment at a particular

site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 10 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 22. Two-way dendrogram from cluster analysis of resident mussel PAH concentrations among sites in 2013. Relative PAH concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the particulate sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 10 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 23. Scatterplot of site-specific mean total mussel density (all species combined) versus total PAH concentrations measured in resident mussel tissues in 2012 and 2013. Mussel density data were provided by Krstolic et al. 2013, Jones et al. 2014, and J. Jones personal communication. Sites are labeled with three letter abbreviations as follows: ART=Artrip, CAR=Carterton, CC=Copper Creek, CLE=Cleveland, CHP=Clinchport, DC=Dump's Creek, GR=Guest River, HF=Horton Ford, IC=Indian Creek, PEN=Pendleton, SIM=Simones, WB=Wallen Bend.

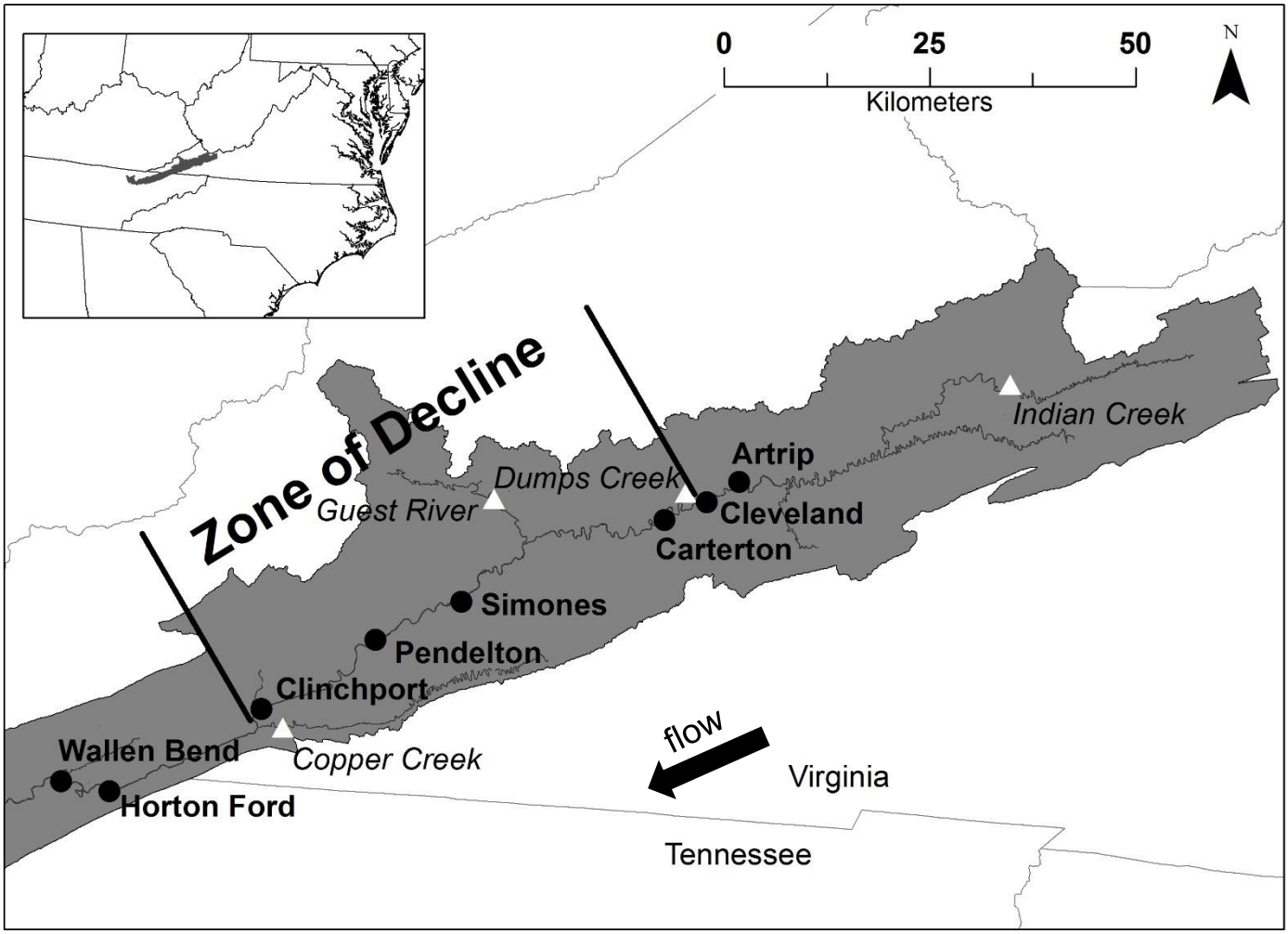
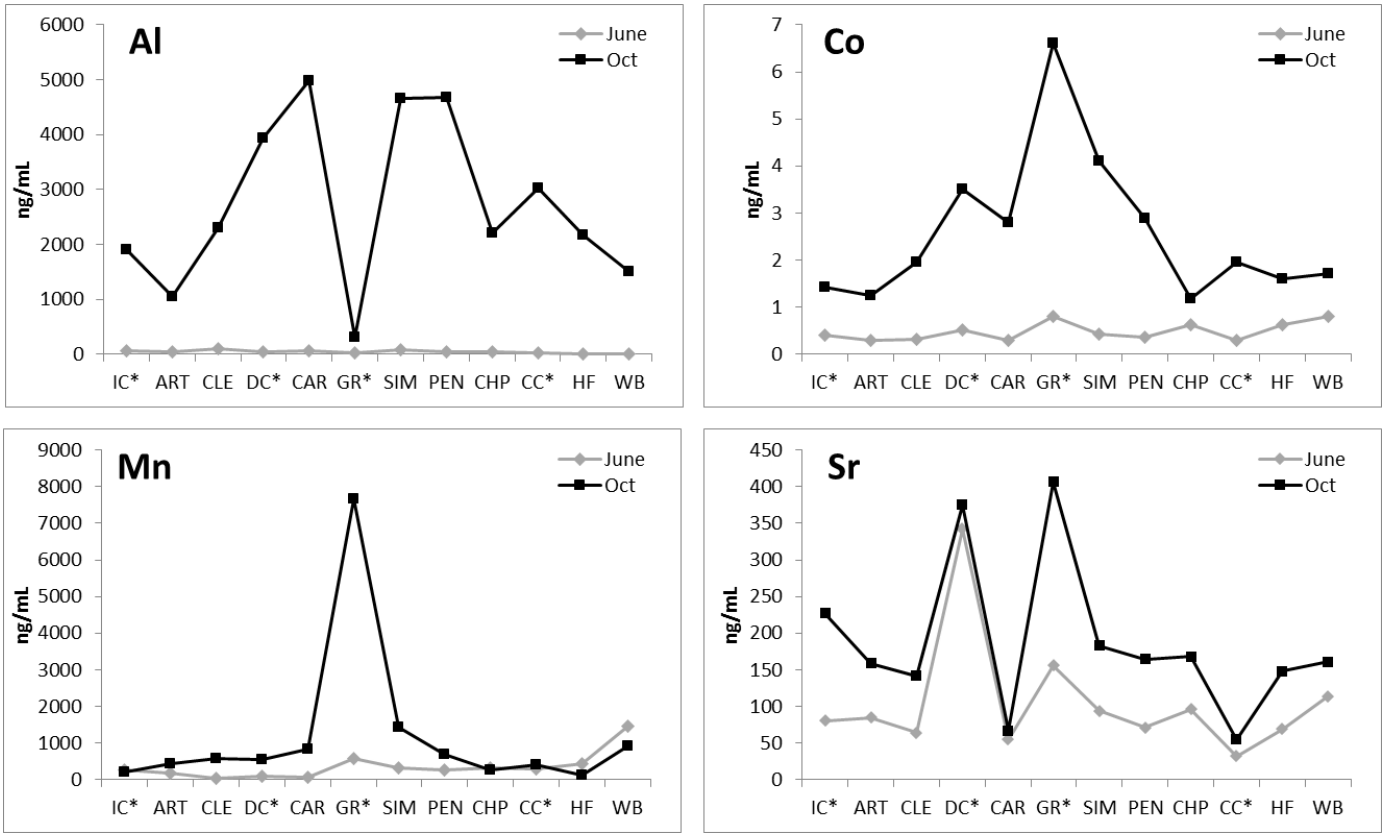

0
25
50
Kilometers
N
Zone of Decline
Guest River
Dumps Creek
Indian Creek
Artrip
Cleveland
Carterton
Simones
Pendelton
Clinchport
Copper Creek
Wallen Bend
Horton Ford
flow
Virginia
Tennessee

Figure 1.


Al
June
Oct
ng/mL
6000
5000
4000
3000
2000
1000
0
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB
Co
June
Oct
ng/mL
7
6
5
4
3
2
1
0
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB
Mn
June
Oct
ng/mL
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB
Sr
June
Oct
ng/mL
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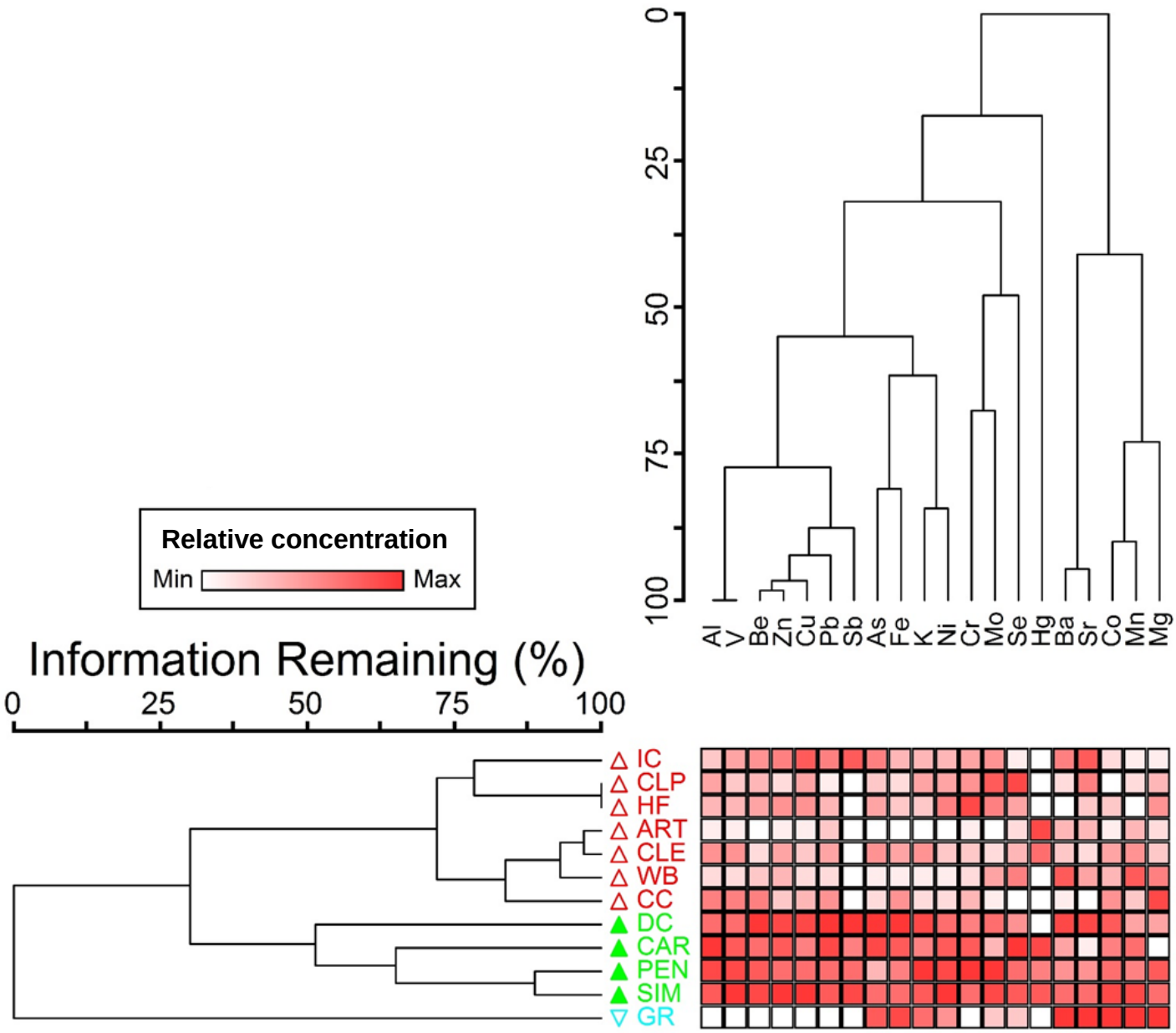
400
350
300
250
200
150
100
50
0
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB

Figure 2.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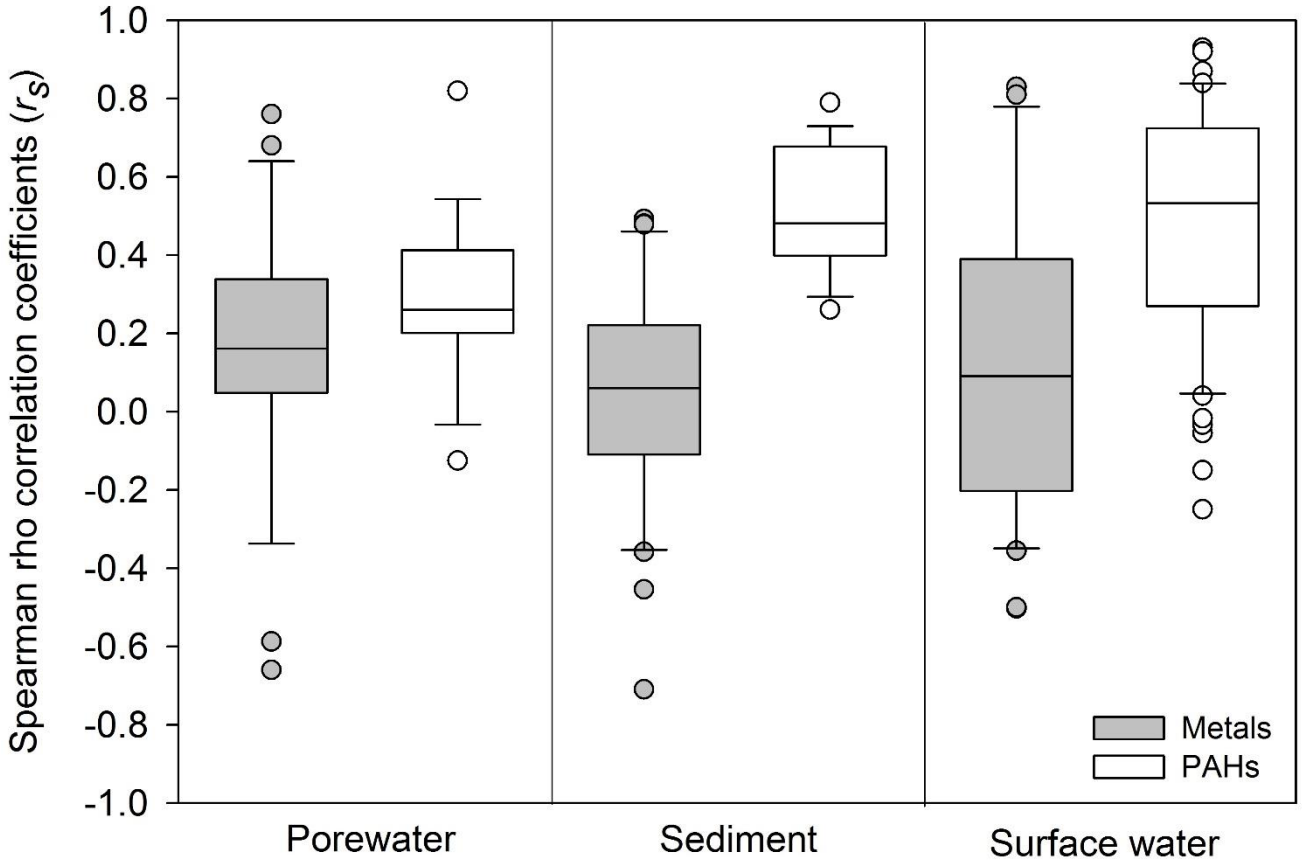
75
100
Al
V
Be
Zn
Cu
Pb
Sb
As
Fe
K
Ni
Cr
Mo
Se
Hg
Ba
Sr
Co
Mn
Mg
Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
IC
CLP
HF
ART
CLE
WB
CC
DC
CAR
PEN
SIM
GR

Figure 3.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0.6
0.4
0.2
0.0
-0.2
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-0.6
-0.8
-1.0
Porewater
Sediment
Surface water
Metals
PAHs

Figure 4.

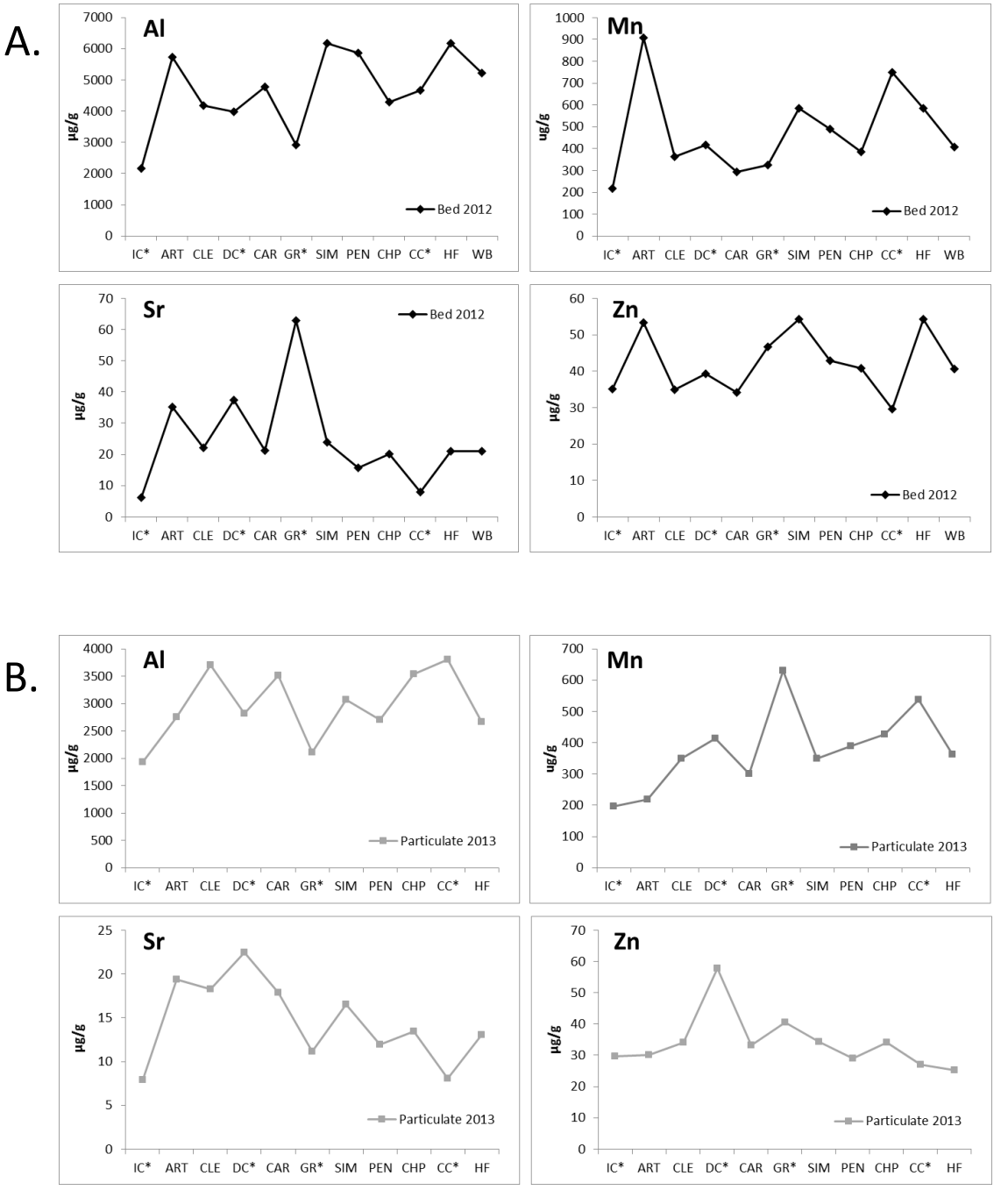
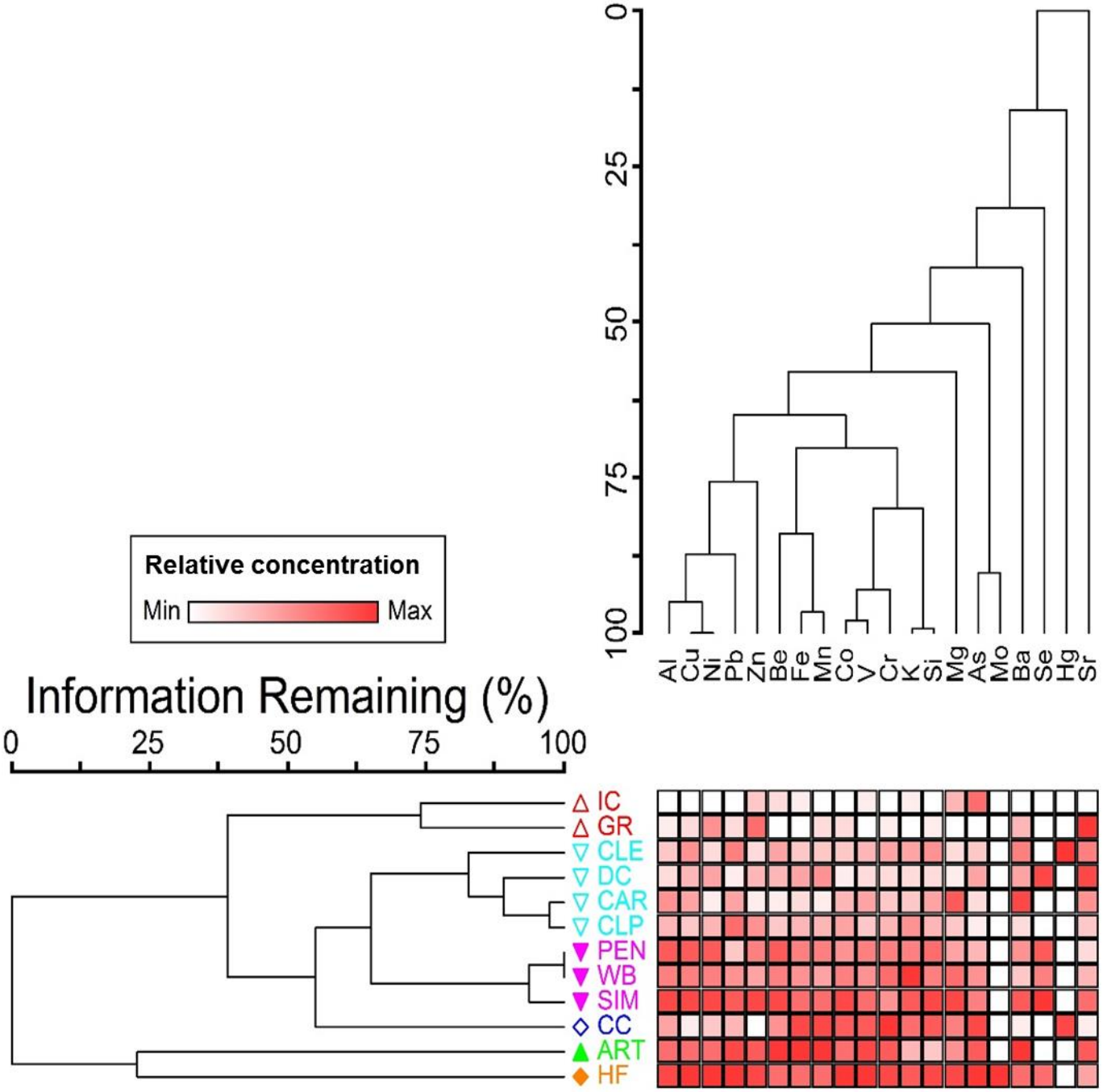

A.
Al
Mn
Sr
Zn
µg/g
ug/g
Bed 2012
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB
B.
Particulate 2013
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF

Figure 5.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Min
Max
Information Remaining (%)
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25
50
75
100
IC
GR
CLE
DC
CAR
CLP
PEN
WB
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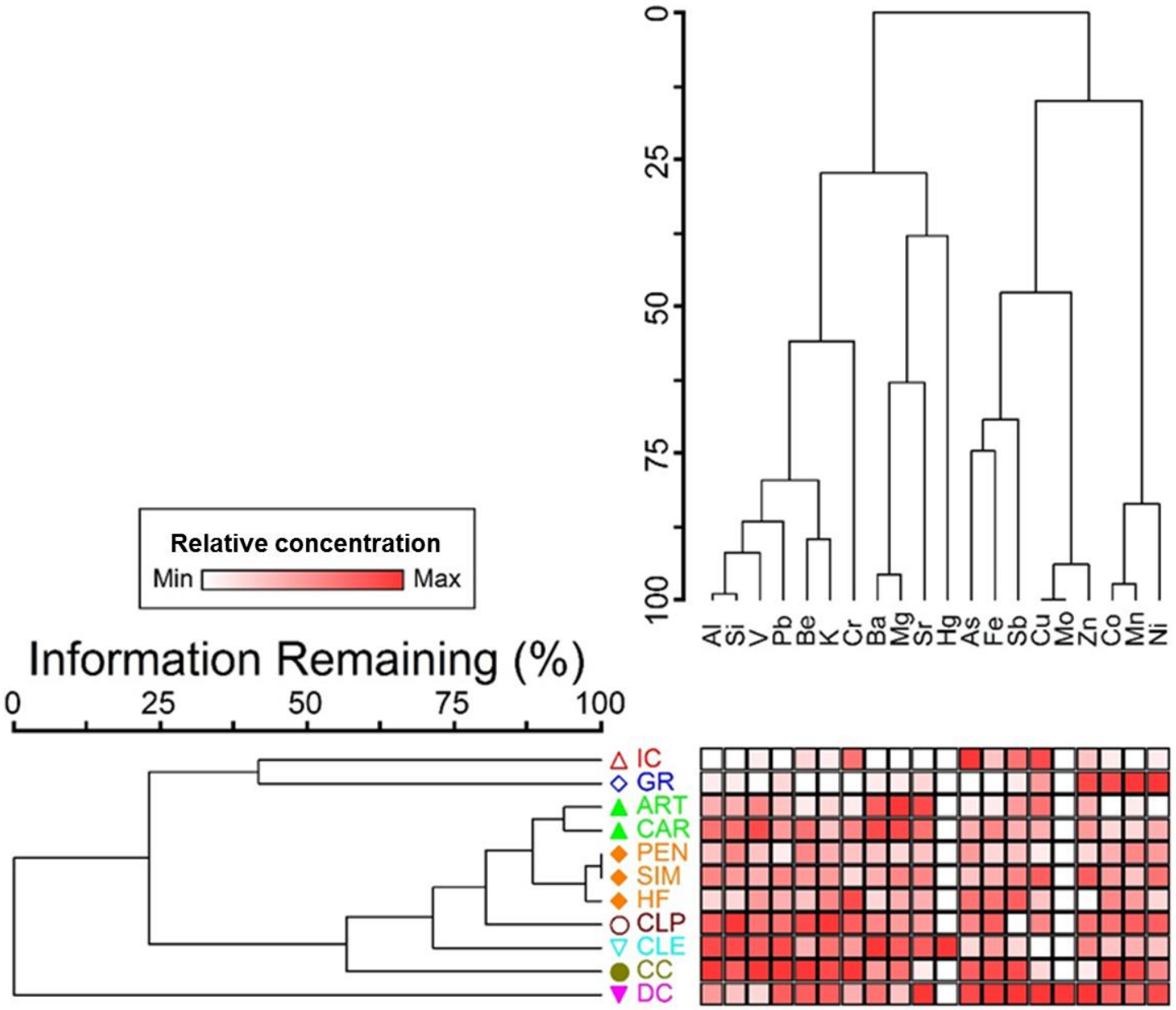
CC
ART
HF
Al
Cu
Ni
Pb
Zn
Be
Fe
Mn
Co
V
Cr
K
Si
Mg
As
Mo
Ba
Se
Hg
Sr

Figure 6.


Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
IC
GR
ART
CAR
PEN
SIM
HF
CLP
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CC
DC
Al
Si
V
Pb
Be
K
Cr
Ba
Mg
Sr
Hg
As
Fe
Sb
Cu
Mo
Zn
Co
Mn
Ni

Figure 7.

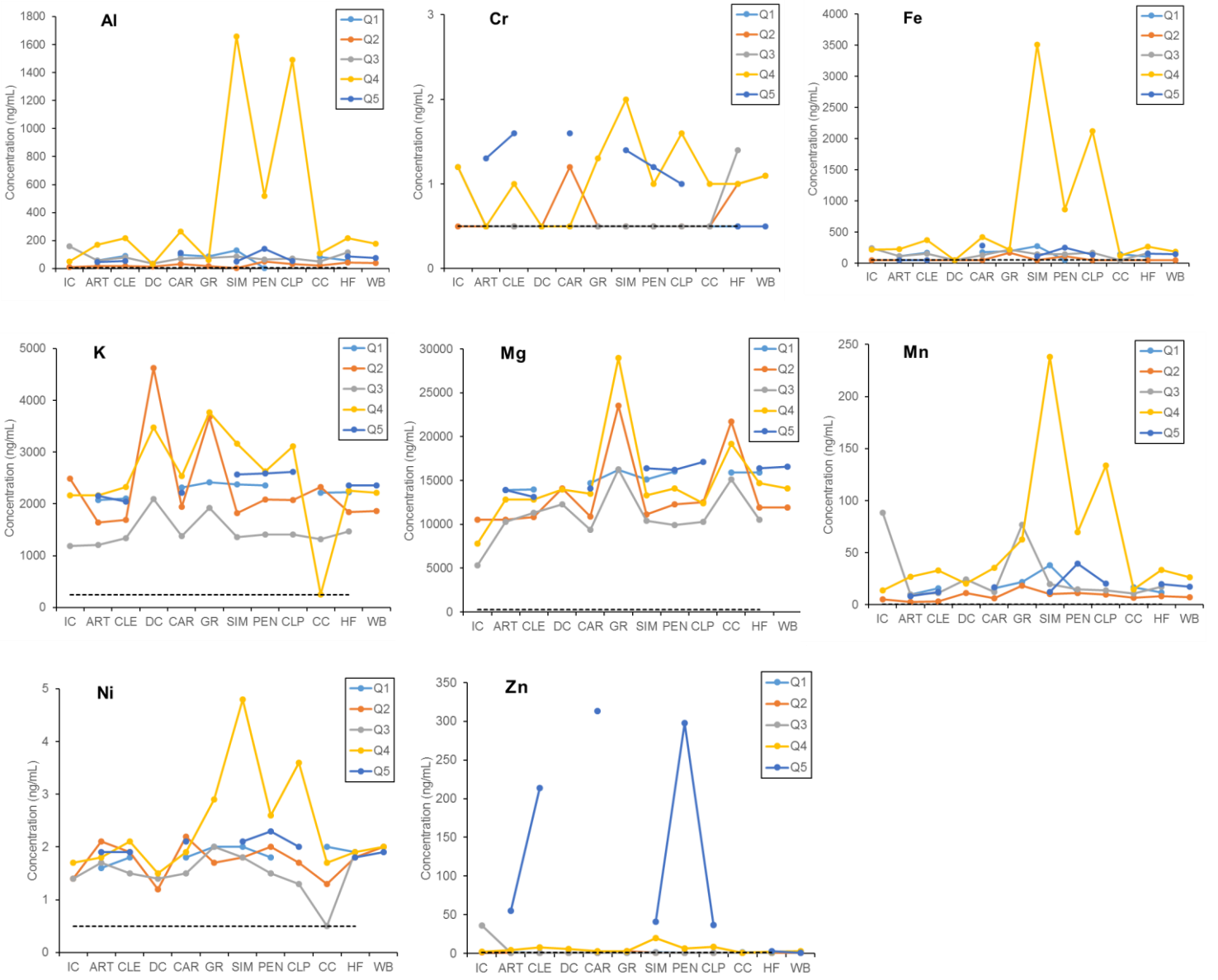

Al
Cr
Fe
K
Mg
Mn
Ni
Zn
Q1
Q2
Q3
Q4
Q5
Concentration (ng/mL)
IC ART CLE DC CAR GR SIM PEN CLP CC HF WB

Figure 8.

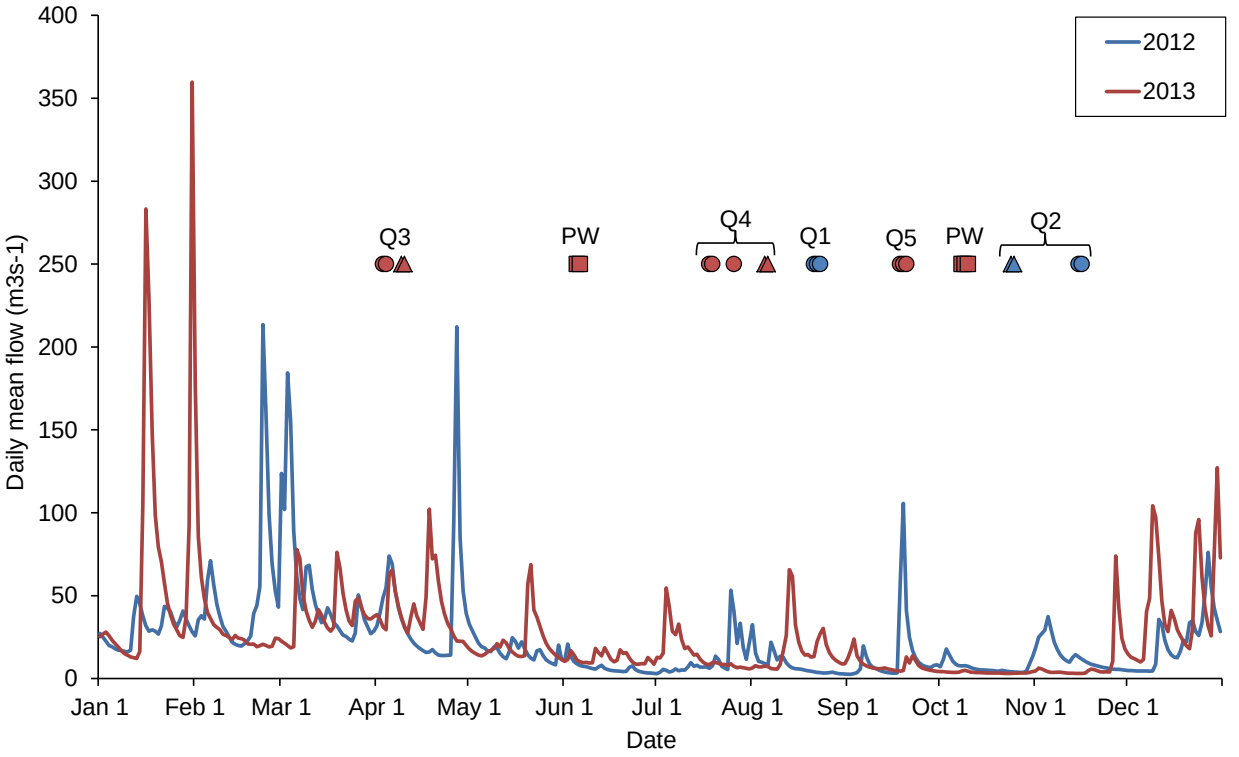

400
350
300
250
200
150
100
50
0
Daily mean flow (m3s-1)
2012
2013
Q3
PW
Q4
Q1
Q5
PW
Q2
Jan 1
Feb 1
Mar 1
Apr 1
May 1
Jun 1
Jul 1
Aug 1
Sep 1
Oct 1
Nov 1
Dec 1
Date

Figure 9.

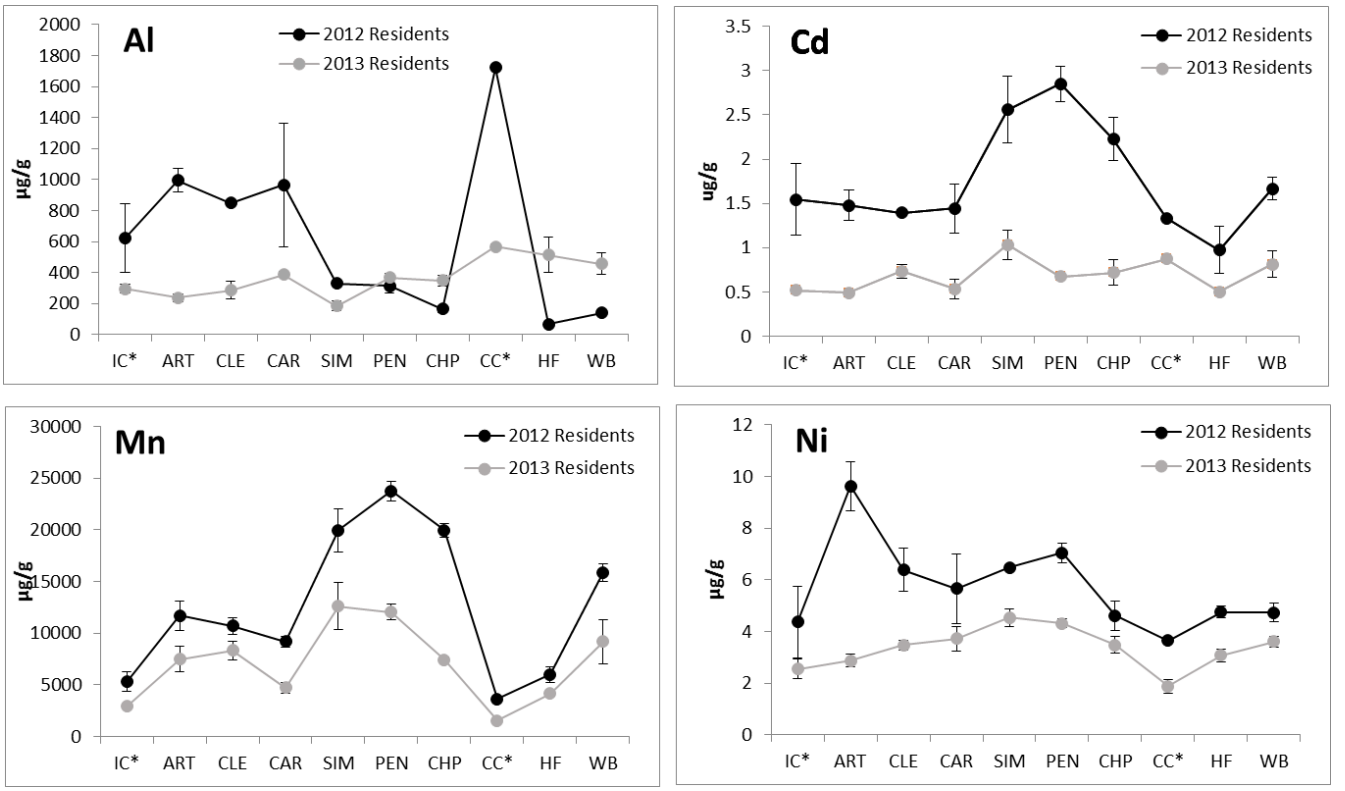

Al
2012 Residents
2013 Residents
µg/g
2000
1800
1600
1400
1200
1000
800
600
400
200
0
IC* ART CLE CAR SIM PEN CHP CC* HF WB
Cd
2012 Residents
2013 Residents
ug/g
3.5
3
2.5
2
1.5
1
0.5
0
IC* ART CLE CAR SIM PEN CHP CC* HF WB
Mn
2012 Residents
2013 Residents
µg/g
30000
25000
20000
15000
10000
5000
0
IC* ART CLE CAR SIM PEN CHP CC* HF WB
Ni
2012 Residents
2013 Residents
µg/g
12
10
8
6
4
2
0
IC* ART CLE CAR SIM PEN CHP CC* HF WB

Figure 10.

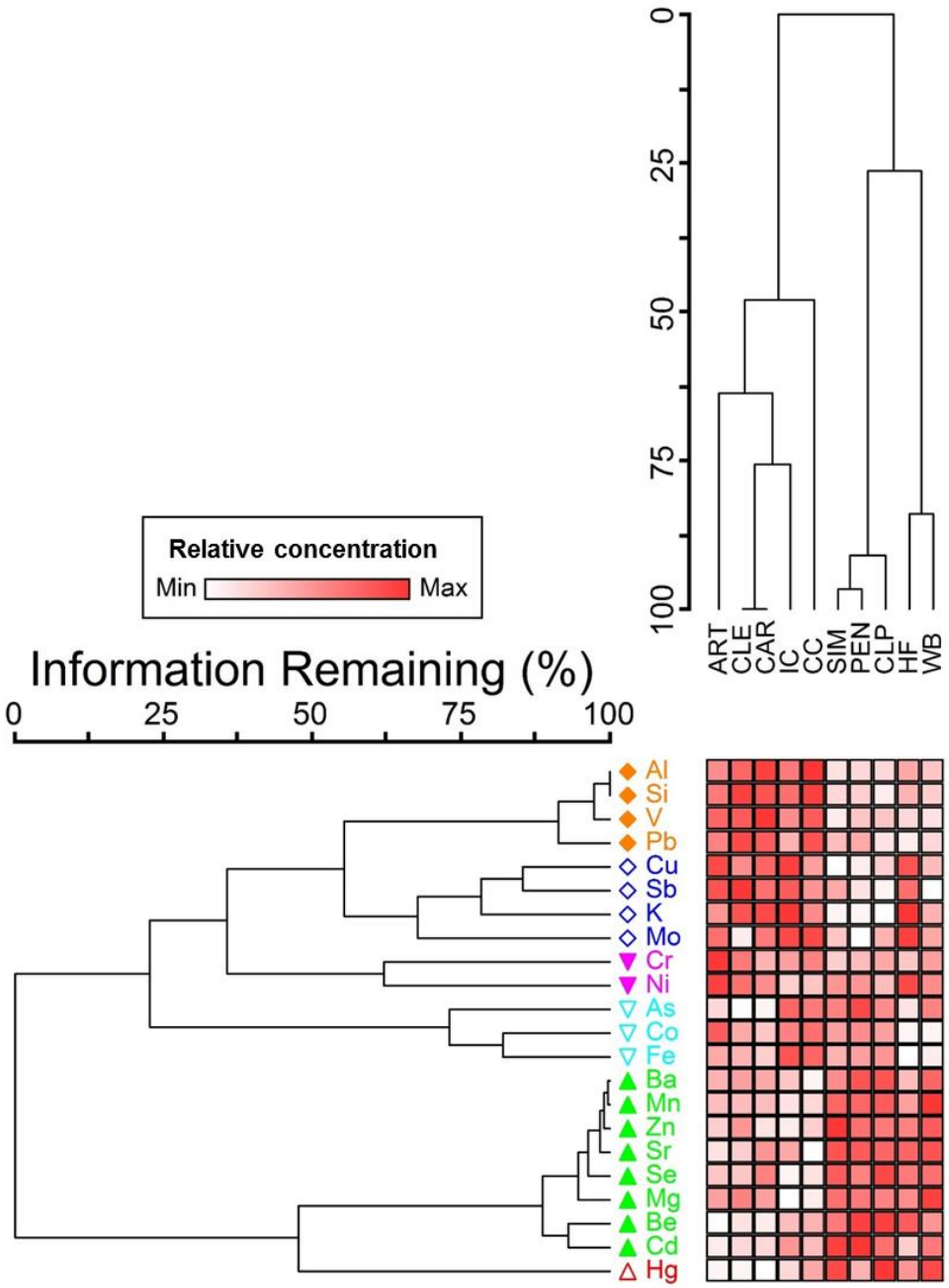

Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
ART
CLE
CAR
IC
CC
SIM
PEN
CLP
HF
WB
Al
Si
V
Pb
Cu
Sb
K
Mo
Cr
Ni
As
Co
Fe
Ba
Mn
Zn
Sr
Se
Mg
Be
Cd
Hg

Figure 11.

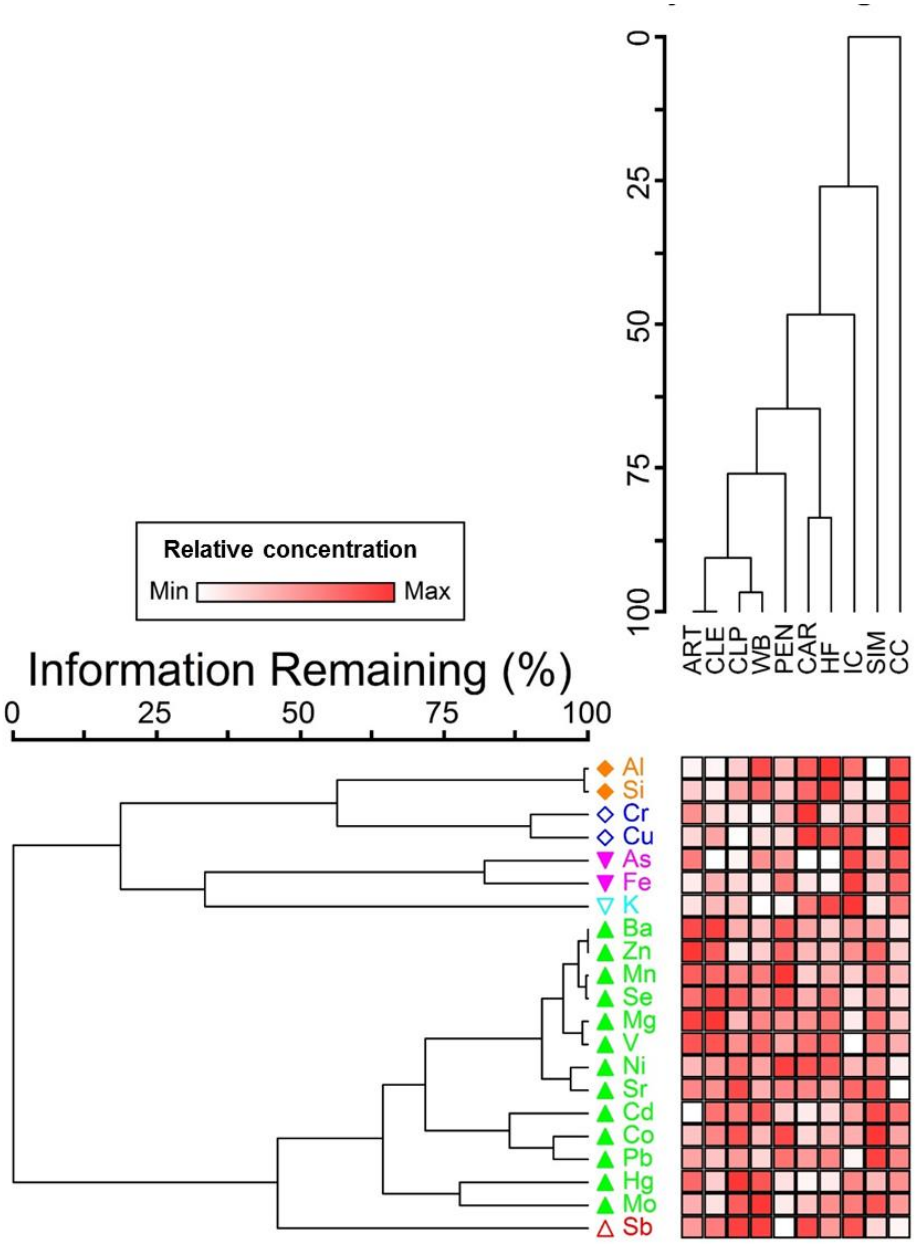

Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
Al
Si
Cr
Cu
As
Fe
K
Ba
Zn
Mn
Se
Mg
V
Ni
Sr
Cd
Co
Pb
Hg
Mo
Sb
ART
CLE
CLP
WB
PEN
CAR
HF
IC
SIM
CC

Figure 12.

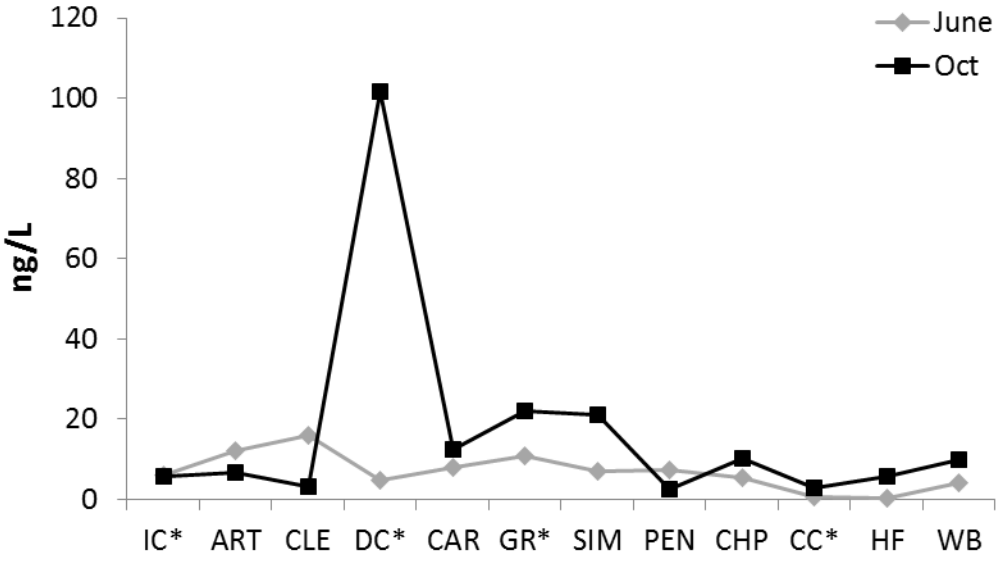

120
100
80
60
40
20
0
ng/L
June
Oct
IC*
ART
CLE
DC*
CAR
GR*
SIM
PEN
CHP
CC*
HF
WB

Figure 13.

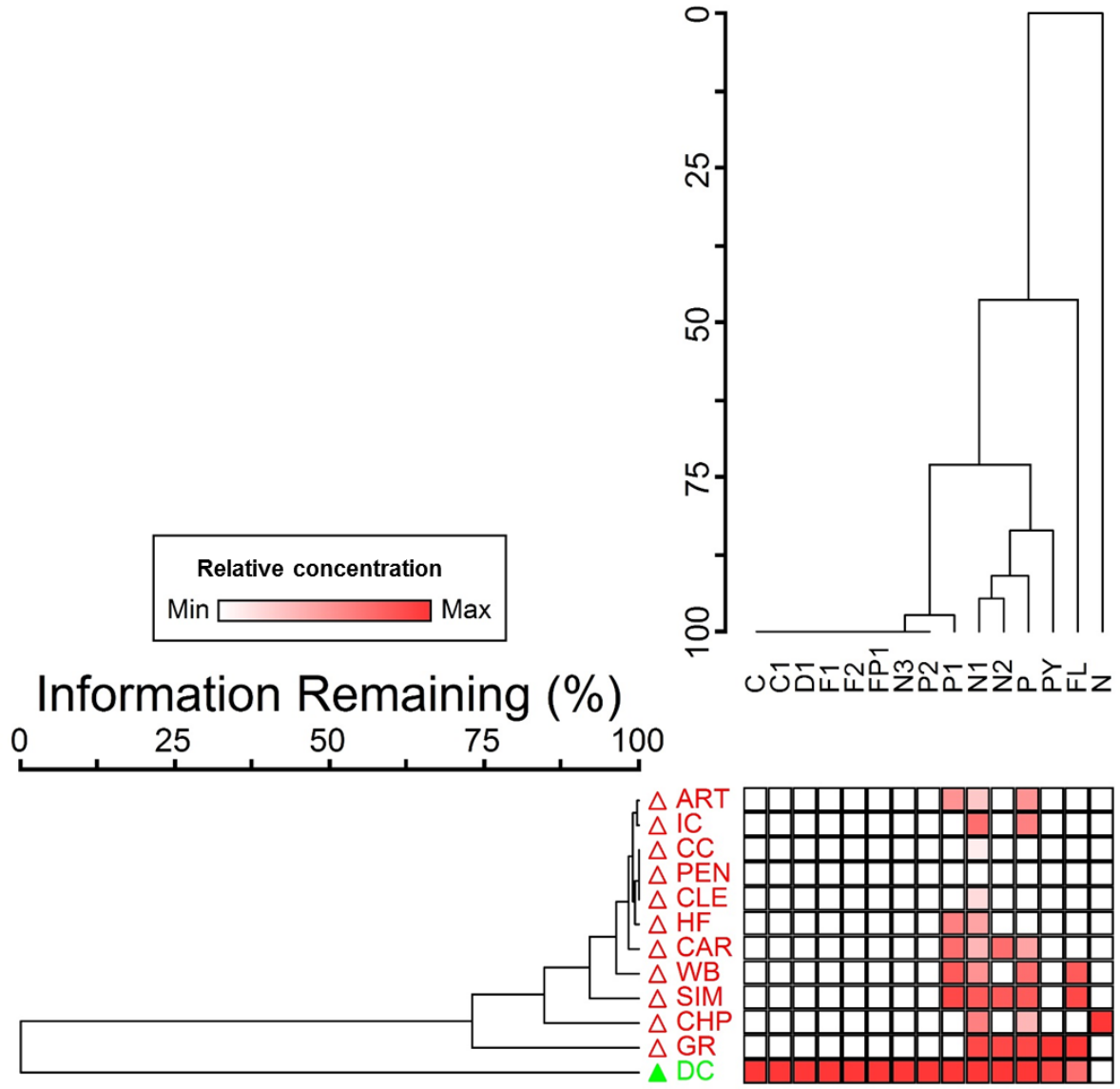

Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
ART
IC
CC
PEN
CLE
HF
CAR
WB
SIM
CHP
GR
DC
C
C1
D1
F1
F2
FP1
N3
P2
P1
N1
N2
P
PY
FL
N

Figure 14.

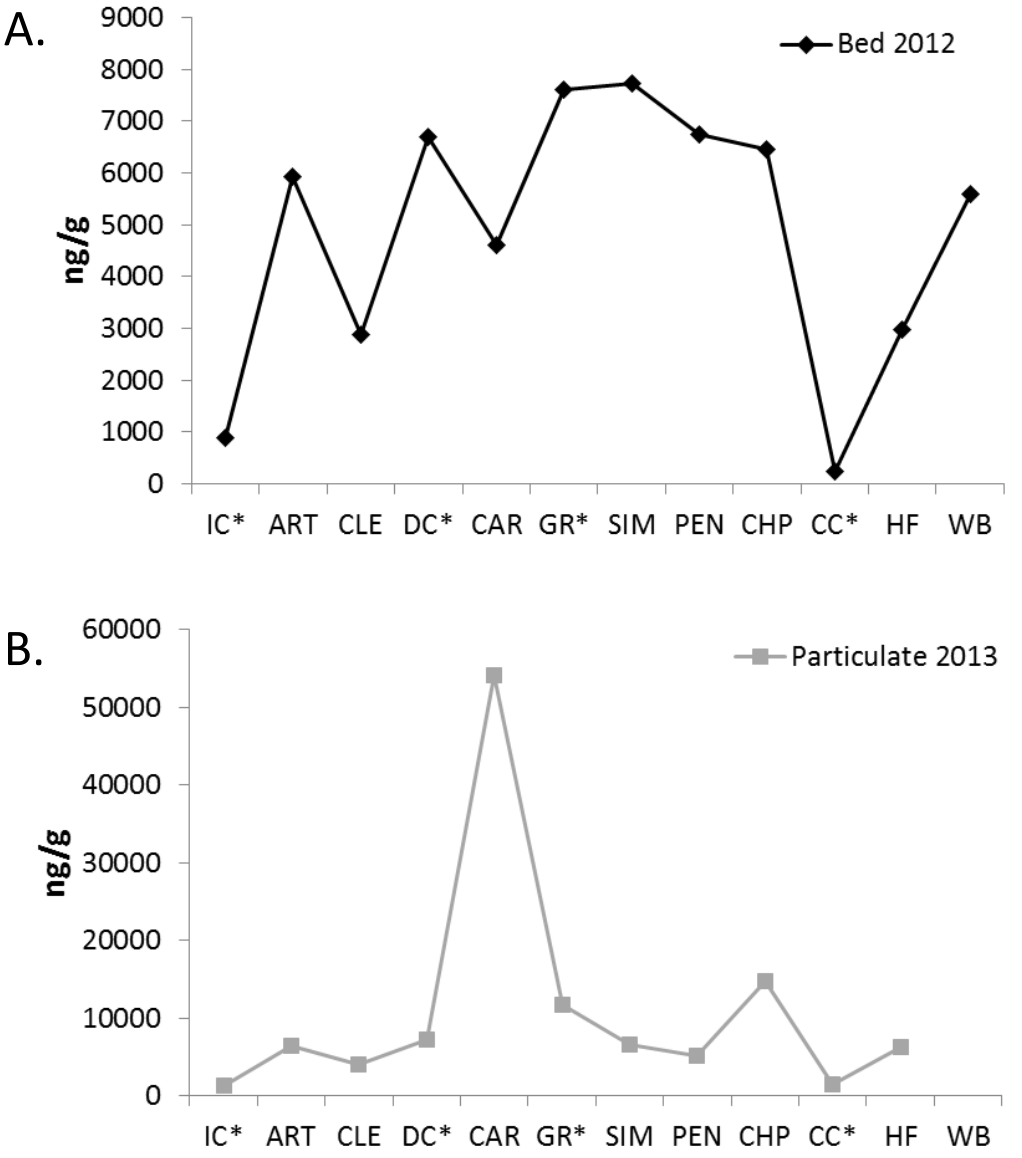

A.
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
ng/g
Bed 2012
IC*
ART
CLE
DC*
CAR
GR*
SIM
PEN
CHP
CC*
HF
WB
B.
60000
50000
40000
30000
20000
10000
0
ng/g
Particulate 2013
IC*
ART
CLE
DC*
CAR
GR*
SIM
PEN
CHP
CC*
HF
WB

Figure 15.

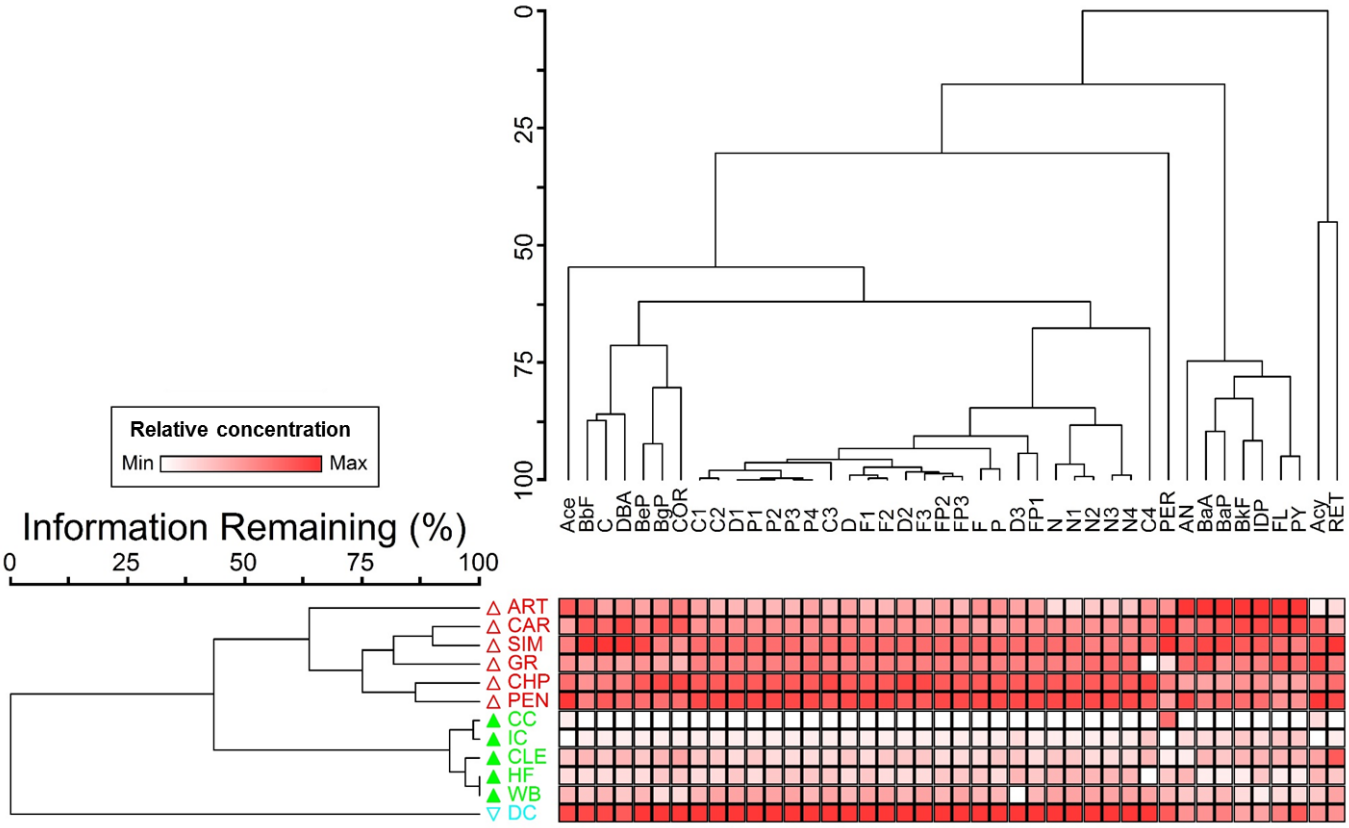

Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
Ace
BbF
C
DBA
BeP
BgP
COR
C1
C2
D1
P1
P2
P3
P4
C3
D
F1
F2
D2
F3
FP2
FP3
F
P
D3
FP1
N
N1
N2
N3
N4
C4
PER
AN
BaA
BaP
BkF
IDP
FL
PY
Acy
RET
ART
CAR
SIM
GR
CHP
PEN
CC
IC
CLE
HF
WB
DC

Figure 16.

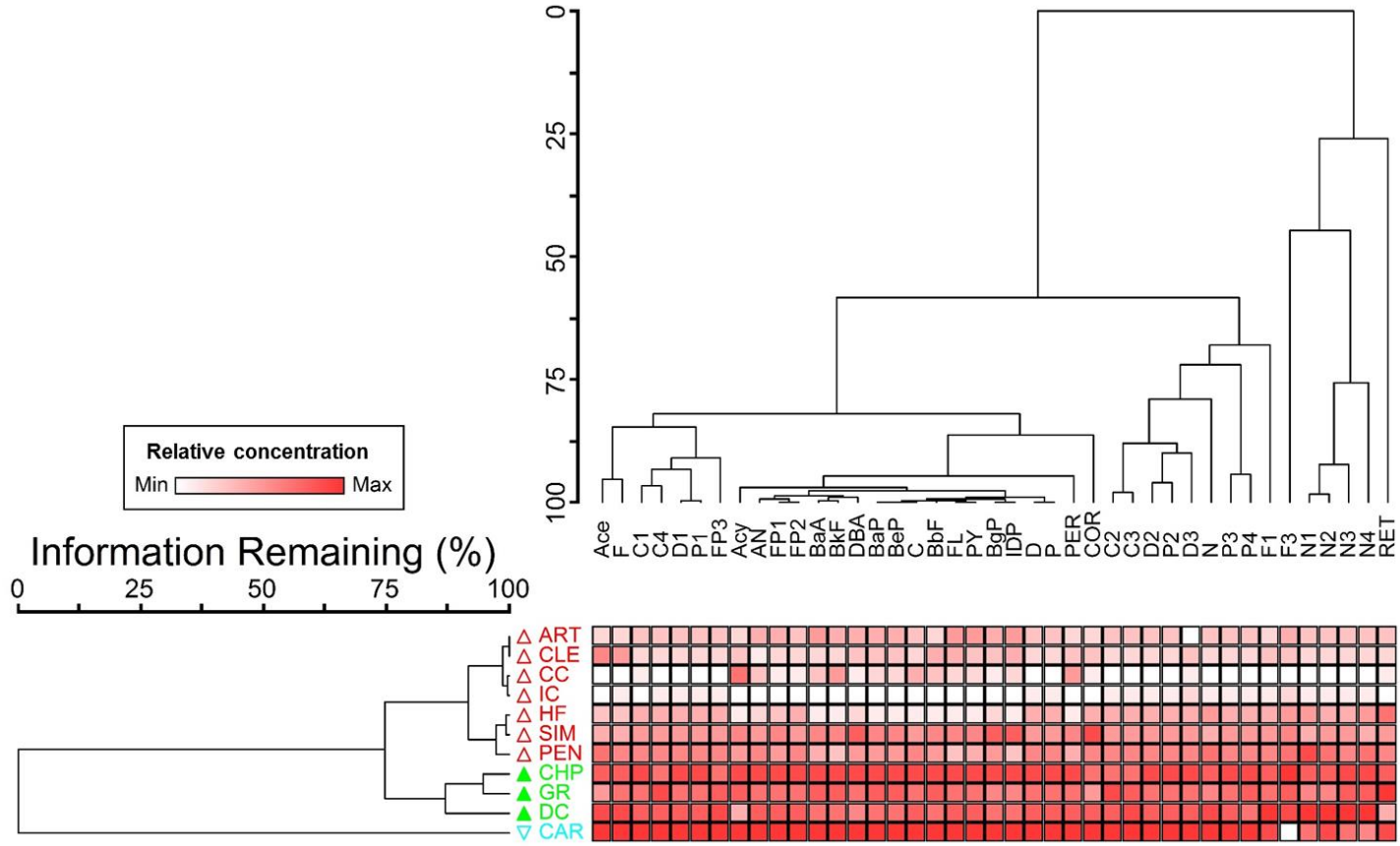

Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
Ace
F
C1
C4
D1
P1
FP3
Acy
AN
FP1
FP2
BaA
BkF
DBA
BaP
BeP
C
BbF
FL
PY
BgP
IDP
D
P
PER
COR
C2
C3
D2
P2
D3
N
P3
P4
F1
F3
N1
N2
N3
N4
RET
ART
CLE
CC
IC
HF
SIM
PEN
CHP
GR
DC
CAR

Figure 17.

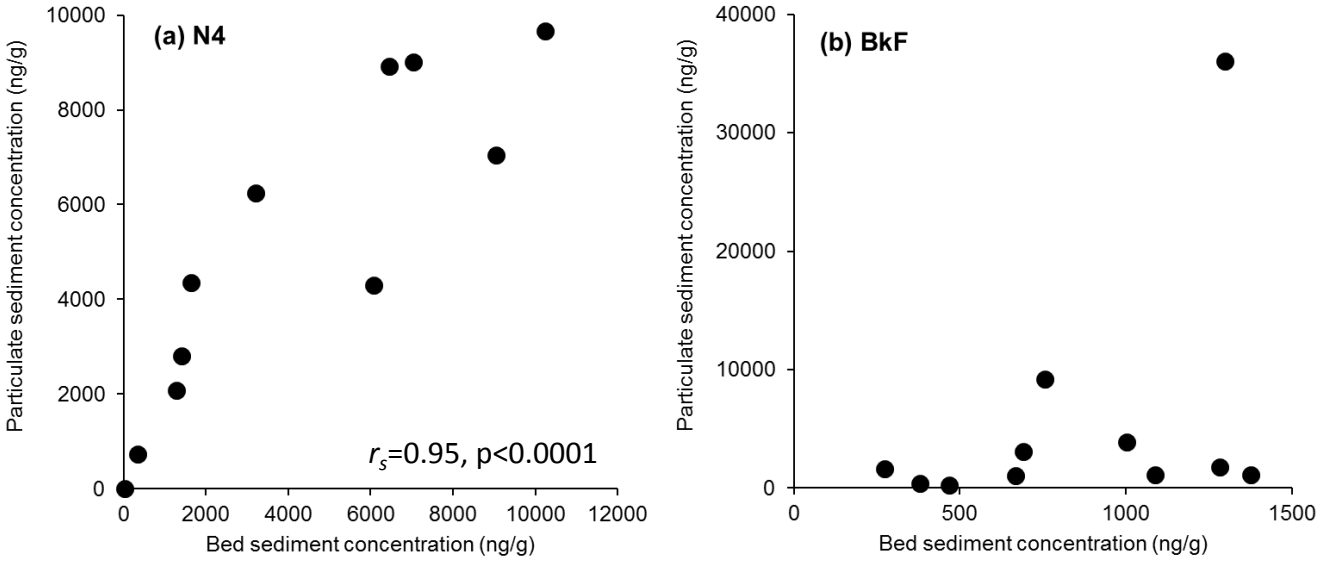

(a) N4
Particulate sediment concentration (ng/g)
Bed sediment concentration (ng/g)
r_s=0.95, p<0.0001
0
2000
4000
6000
8000
10000
12000
(b) BkF
Particulate sediment concentration (ng/g)
Bed sediment concentration (ng/g)
0
10000
20000
30000
40000
0
500
1000
1500

Figure 18.

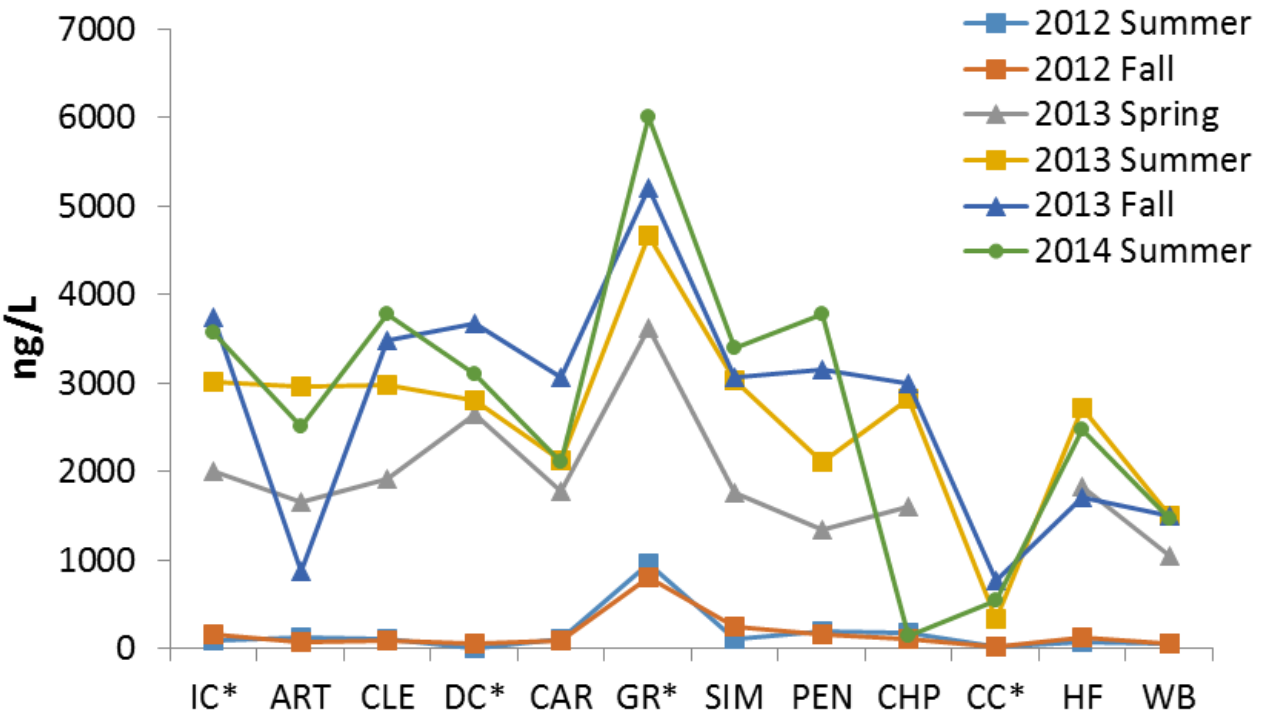

7000
6000
5000
4000
3000
2000
1000
0
ng/L
IC*
ART
CLE
DC*
CAR
GR*
SIM
PEN
CHP
CC*
HF
WB
2012 Summer
2012 Fall
2013 Spring
2013 Summer
2013 Fall
2014 Summer

Figure 19.

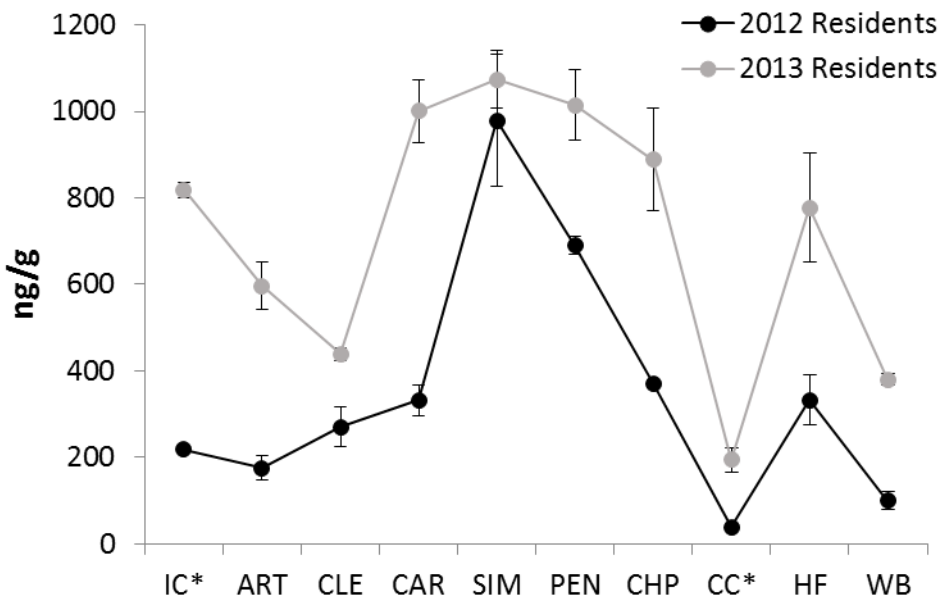

1200
1000
800
600
400
200
0
ng/g
2012 Residents
2013 Residents
IC*
ART
CLE
CAR
SIM
PEN
CHP
CC*
HF
WB

Figure 20.

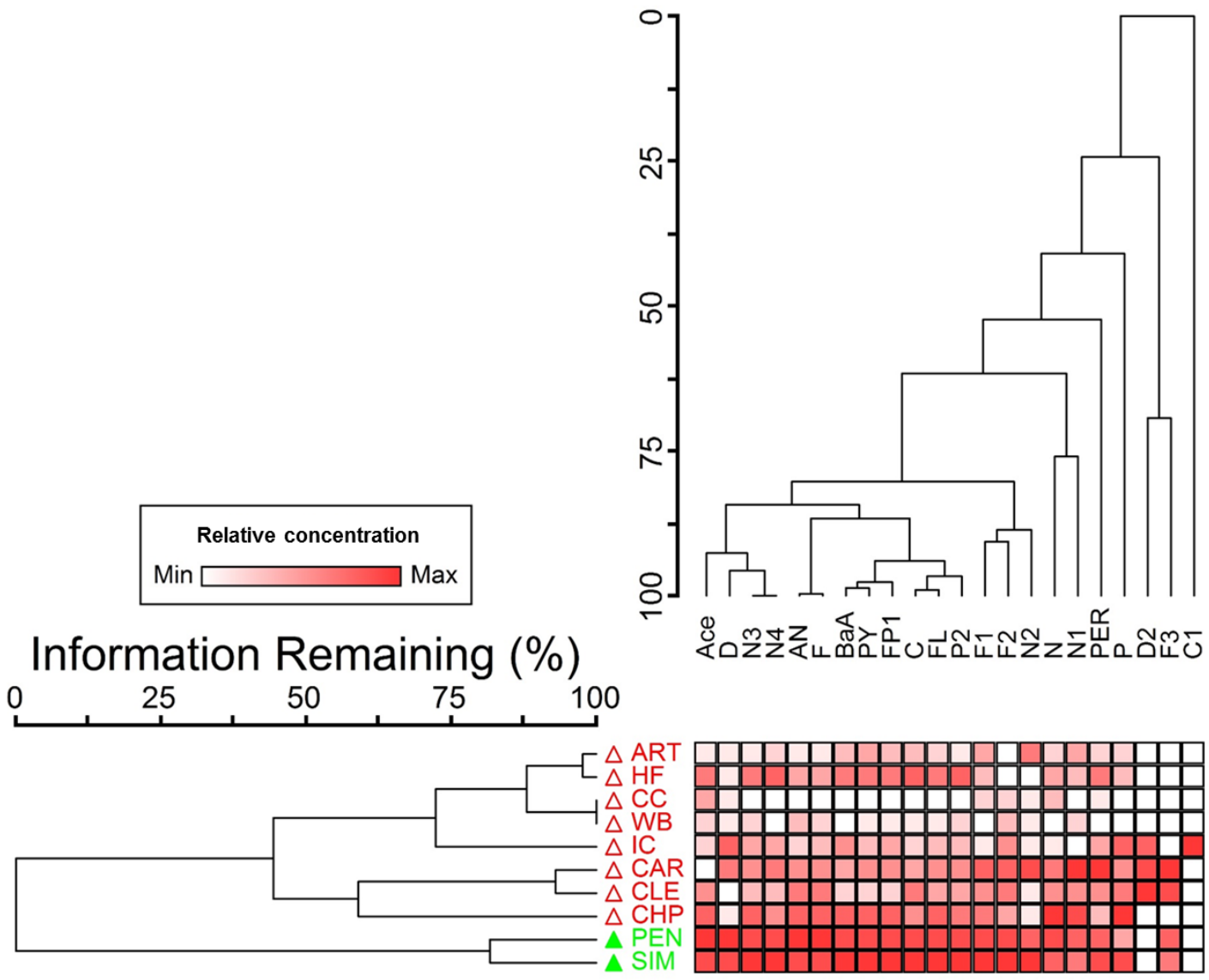

Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
ART
HF
CC
WB
IC
CAR
CLE
CHP
PEN
SIM
Ace
D
N3
N4
AN
F
BaA
PY
FP1
C
FL
P2
F1
F2
N2
N
N1
PER
P
D2
F3
C1

Figure 21.

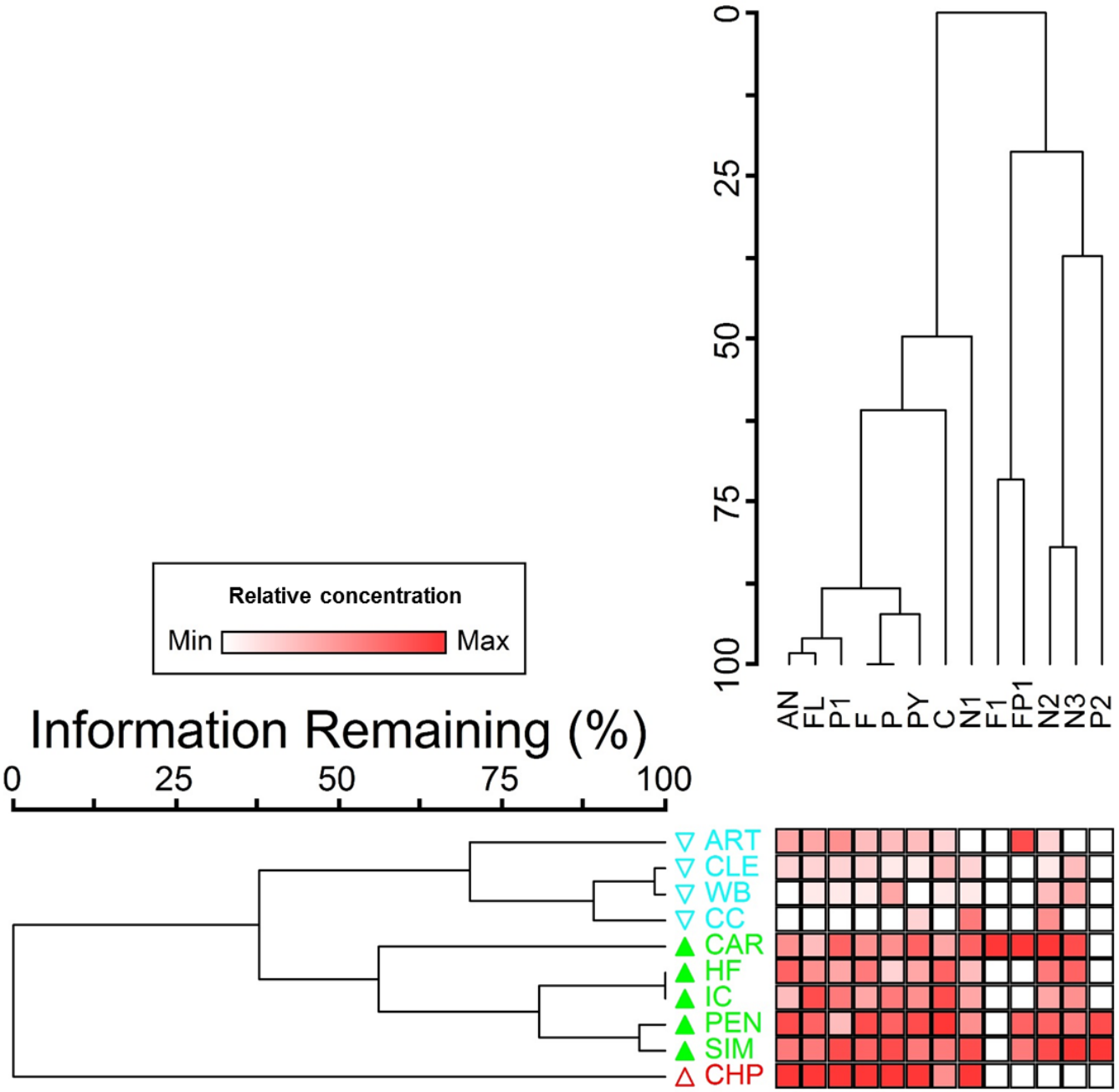
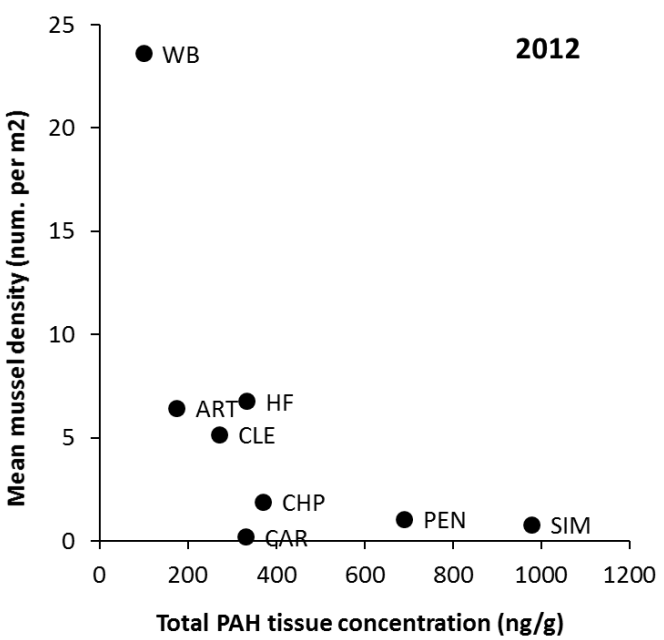

Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
ART
CLE
WB
CC
CAR
HF
IC
PEN
SIM
CHP
AN
FL
P1
F
P
PY
C
N1
F1
FP1
N2
N3
P2

Figure 22.


2012
Mean mussel density (num. per m2)
Total PAH tissue concentration (ng/g)
WB
ART
HF
CLE
CHP
CAR
PEN
SIM
0
5
10
15
20
25
0
200
400
600
800
1000
1200

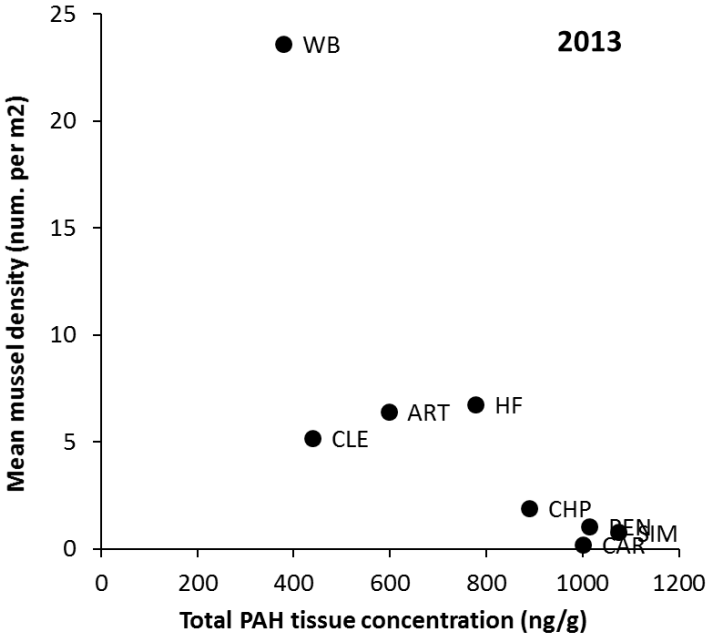

2013
Mean mussel density (num. per m2)
Total PAH tissue concentration (ng/g)
WB
ART
HF
CLE
CHP
PEN
SIM
CAR
0
5
10
15
20
25
0
200
400
600
800
1000
1200

Figure 23.

Chapter 3: Spatial variation in growth and survival of juvenile freshwater mussels in the Clinch River, USA: relations to contaminant exposure and feeding ecology

Abstract

Mussel populations in portions of the Clinch River in Virginia and Tennessee have declined substantially in recent decades, but the causes of the decline remain unknown. We evaluated the effects of inorganic and organic contaminant exposure on the growth and survival of juvenile freshwater mussels (*Villosa iris* in 2012 and *Lampsilis fasciola* in 2013) deployed at 12 sites in the Clinch River and its tributaries, which spanned a wide range of mussel density and diversity. We used two *in situ* deployment techniques; cages filled with ambient stream bed sediment and enclosures (silos) positioned at the sediment-water interface. In the two consecutive years of the study, we found that over a 5-6 month deployment period, mussel survival was generally high at most sites, but growth rate varied by site and was negatively correlated with tissue concentrations of certain mining-associated metals, especially in caged mussels in 2012. Growth rate also differed between deployment methods and was considerably greater for juvenile mussels in cages than in silos between years. Substantial amounts of polycyclic aromatic hydrocarbons (PAHs) accumulated in the juvenile mussels over the relatively brief deployment period and were similar to concentrations measured in resident mussels from the same sites (see Chapter 2). Moreover, PAH concentrations in juvenile mussels were strongly correlated with native mussel density, but not with metal tissue concentrations. These findings suggest that PAHs may exert chronic, potentially lethal effects on mussels, whereas certain metals, when present at high enough concentrations, can have sublethal effects through depressed growth.

Introduction

Native freshwater mussel populations have experienced severe declines and are one of the most rapidly declining faunal groups in North America. Approximately 70% of the nearly 300 freshwater mussel species found in North America are listed as endangered, threatened, or of special concern (Williams et al. 1993). While mussel declines due to site-specific, catastrophic events have been identified, it is more difficult to find the causes of the gradual loss of freshwater mussel abundance and diversity (Bogan 1993; Strayer et al. 2004; Lydeard et al. 2004). Many anthropogenic stressors associated with human activities have been identified as potential factors, but contaminant pollution from point and non-point sources is often cited as contributing to the declines (Richter et al. 1997; Augspurger et al. 2007; Cope et al. 2008; Downing et al. 2010). Freshwater mussels are potentially exposed to contaminants from various sources and routes of uptake at several developmental stages due to their unique life cycle; namely, exposure from tissue of the host fish in the larval stage (glochidia), to stream sediment and pore water while burrowed and primarily pedal feeding as young juveniles, and through the water column by filter-feeding surface water at the sediment/water interface as older juveniles and adults (reviewed in Cope et al. 2008). Furthermore, several studies have indicated that early life stages are especially sensitive to some contaminants compared to other aquatic species that are routinely tested (Milam et al. 2005) such as ammonia (Augspurger et al. 2003, Mummert et al. 2003), metals (Jacobson et al. 1997, Besser et al. 2015; Wang et al. 2010), pesticides (Conners and Black. 2004; Bringolf et al. 2007a, 2007b, 2007c), and polycyclic aromatic hydrocarbons (PAHs; Wang et al. 2013). This sensitivity of early life stages may contribute to

mussel declines by reducing fitness and recruitment (Cope et al. 2008; Bruenderman and Neves 1993; Neves and Widlak 1987).

The Clinch River watershed of the upper Tennessee River basin of Virginia and Tennessee is an example of a watershed that has experienced both known and unknown causes of mussel declines in the past several decades. This watershed supports one of North America's greatest concentrations of freshwater biodiversity (Neves et al. 1997) with 46 extant mussel species, 20 of which are federally endangered, in addition to 5 federally threatened and endangered fishes (Jones et al. 2014). Significant declines in both diversity and abundance have been observed in mussel populations in certain reaches of the river. Notably, an 88-km reach of the river from downstream of the town of Carbo, Virginia (USA; river km 431) to Clinchport, Virginia (USA; river km 343) is considered a mussel "zone of decline" (ZOD; Figure 1). Within the ZOD, mussel densities have decreased more than 70% from 1979 to 2004 (Jones et al. 2014). Interestingly, both upstream and downstream of the ZOD mussel populations are stable or increasing (i.e., numerous adult cohorts and evidence of recent recruitment; Jones et al. 2014). This contrast in the status of mussel populations in the Clinch River provides an excellent opportunity to study the potential factors involved in freshwater mussel declines.

The overall aim of this study was to evaluate the effects of contaminant (inorganic and organic) exposure on growth and survival of juvenile mussels in the Clinch River and its tributaries, which spanned a range of mussel abundances, including those within the documented ZOD, to provide insight into the potential role of contaminants in the decline of mussels in the system. In the study, mussels were held in cages filled with ambient stream bed sediment (exposure to sediment and sediment pore water) and in silos sitting atop the stream bottom (exposure to the water column) for five to six months in two consecutive years (different species

in each year) to determine whether contaminant accumulation differed between these two feeding habitats and exposure routes. The simultaneous use of cages and silos has not been previously evaluated; therefore, our study provides new insight into the utility of this approach. Because juvenile and adult mussels have limited mobility, deployed juvenile mussels are indicators of local environmental conditions and exposure, providing natural resource managers, conservation agencies, and decision makers with the information (along with our results on resident mussels; see Chapter 2) needed to better assess the role of contaminants in the decline of mussels in the Clinch River.

Methods

Study Area

Figure 1 (Refer to Chapter 2 for details).

Field Deployment and Collection

To assess the accumulation and response of mussels to contaminant exposure, we deployed juvenile mussels in sediment cages at the 8 mainstem sites and in water column silos (Barnhart et al. 2007) at all 12 sites (8 mainstem and 4 tributary sites). At each site, six silos and six cages (mainstem only) were placed in habitats with a substrate mixture of gravel and sand (Ostby et al. 2014) and in locations of historic mussel populations based on published literature and expert consensus (Jones et al. 2014).

The cages were constructed from polyethylene industrial containers with mesh sides and base (406.4 mm x 304.8 mm x 114.3 mm; Schaefer Systems, 52356, US Plastic Corporation, Lima, Ohio) with plastic mesh (6.34 mm) affixed to the inside of the cages on the top, sides, and bottom to contain mussels and allow water to flow through the cages (Figure 2A). We deployed the cages in areas with fine and coarse gravel (~1-60 mm) and used the river substrate to fill the

cages ~3/4 full, allowing mussels to live in their natural habitat, while facilitating ease of recovery. Cages were partially buried to ensure the tops of the cages were flush with the sediment-water interface. The cages were placed in the river the week before mussels were deployed to ensure that any water soluble or volatile chemicals from the construction materials were removed from the cages (ASTM 2002).

Concrete mussel silos were constructed to house mussels in the water column (~6 cm above the sediment-water interface; Barnhart et al. 2007). Mussels were placed in cylindrical chambers (14 cm) with plastic mesh (6 mm mesh size) on the top and bottom to allow water to circulate through the chamber (Figure 2B). Silos and cages were secured to their deployment location by a large (91 cm) nylon cable tie affixed to a 35-cm length section of steel rebar hammered into the substrate just upstream (~15-cm) of the cage or silo.

The mussels used in this study were propagated by standard host fish infection techniques (Barnhart 2006) and grown at the Freshwater Mollusk Conservation Center at Virginia Tech in Blacksburg, VA (*Villosa iris* in 2012 and *Lampsilis fasciola* in 2013) and the Virginia Department of Game and Inland Fisheries Aquatic Wildlife Conservation Center in Marion, VA (*L. fasciola* in 2013) in water recirculating systems using pond water (Carey et al. 2013). *Villosa iris* were ~17 months old and ranged in size from 15-40 mm at the time of deployment. Approximately half of the *L. fasciola* ranged in size from 40-45 mm, while the other half were 20-22 mm, owing to the two source facilities. We used a random number generator (Microsoft Excel) to randomly select 18 (*V. iris* in 2012) and 11 (*L. fasciola* in 2013) juvenile mussels for deployment in each cage and silo for total of 2,160 mussels in 2012 and 1,320 mussels in 2013. Fewer mussels were deployed per cage/silo in 2013 than 2012 to account for the larger mussel sizes used that year in an attempt to achieve equal biomasses per cage/silo between years. At the

same time, a subset of 161 *V. iris* in 2012 and 179 *L. fasciola* in 2013 were randomly set aside and immediately frozen to serve as baseline mussels for tissue contaminant analyses.

In 2012, we deployed *V. iris* on June 5-6, but in 2013, deployment of mussels (*L. fasciola*) was delayed until July 31-August 1 due to a series of storms and subsequent high water levels in the river. Once mussels were deployed, cages and silos were monitored monthly throughout the deployment period, and cleaned by fanning the water directly above the cage or silo to remove any fine sediment deposition. We retrieved *V. iris* from all sites on October 23-25, 2012, and retrieved *L. fasciola* on November 5-7, 2013. Upon collection, mussels were transported on ice to the laboratory where we transferred them to a -20°C freezer for storage. Prior to analyses, mussels were thawed, measured, weighed and dissected; mussel tissue was equally divided (from 3 cages or silos per sample) to create two composite tissue samples per site. The composite tissue samples were stored frozen (-80°C) until chemical analyses.

Laboratory Analyses and Quality Control

Metals and Organics: Refer to Chapter 2.

Stable Isotopes: Samples of baseline juvenile mussels, deployed juvenile mussels, and native resident mussels in 2012 and 2013 were sent to the Cornell University Stable Isotope Laboratory (Ithaca, New York) for carbon and nitrogen stable isotope analyses, which were performed on a Thermo Delta V isotope ratio mass spectrometer interfaced to a NC2500 elemental analyzer. The laboratory routinely calibrates in-house standards against international reference materials provided by the International Atomic Energy Association. To ensure accuracy of analyses, an in-house standard (mink tissue) was analyzed after every 10 samples. For this analytical sample run, the overall standard deviation of the internal standard was 0.13 ‰ for $\delta\ ^{15}N$ and 0.12 ‰ for $\delta\ ^{13}C$ in 2012 and 0.10 ‰ for $\delta\ ^{15}N$ and 0.07 ‰ for $\delta\ ^{13}C$ in 2013. The ability of the instrument to

accurately measure samples across a gradient of amplitude intensities was also tested with a methionine standard. Based on these results, δ values obtained between the amplitudes of 400 mV and 22000 mV for $\delta^{15}N$ had a linearity error of 0.22 ‰ and between 400 mV and 13000 mV for $\delta^{13}C$, error was 0.31 ‰ in 2012 and δ values obtained between the amplitudes of 300 mV and 17000 mV for $\delta^{15}N$ had an error associated with linearity of 0.31 ‰ and between 500 mV and 20000 mV for $\delta^{13}C$, error is 0.11 ‰ in 2013. Isotope corrections were performed with a two-point normalization (linear regression) of all $\delta^{15}N$ and $\delta^{13}C$ using two additional in-house standards (CBT, a trout standard, and HCRN, a corn standard).

Statistical analyses

Recovery and Survival: Not all mussels were recovered from the cages and silos at the time of collection. The unrecovered mussels were recorded as 'missing' and not assumed to be dead. Accordingly, we calculated survival as the percent of mussels recovered alive out of the mussels recovered. We used analysis of variance (ANOVA) to compare survival among sites for each deployment method (cages or silos) when parametric and homogeneity of variance assumptions were satisfied. When these assumptions were not met, the Kruskal-Wallis test was used to compare survival among sites. Paired t-tests were used to compare survival between cages and silos (mainstem sites only) each year.

Growth: The size distribution of mussels deployed in 2013 was bimodal (Figure 3), owing to the different hatchery sources of the two size groups. Disregarding this bimodality and calculating a single mean of the entire distribution would result in a value that falls between the two size modes and would not be representative of mussel lengths. Moreover, it is understood that intra-specific growth rates are highly dependent on body size in many organisms, including mussels (Bailey and Green 1988; Harmon and Joy 1990; Wong et al. 2012; Malathi and Thippeswamy

2013; Reátegui-Zirena et al. 2013). Accordingly, we estimated growth rates separately for each size mode ('small' and 'large' mussels). To assign mussels to their appropriate size group ('large' or 'small', based on their length), we performed modal analyses using the NORMSEP procedure in FiSAT II (version 1.2.2, Food and Agriculture Organization of the United Nations, Rome, Italy). Modal analyses were conducted for initial and final length distributions for each site using data pooled across samples within a site. Separation index (SI) values from NORMSEP ranged from 4.2 to 7.9 (mean = 6.2), well above the threshold considered as 'reliable separation' (SI > 2, Bhattacharya 1976), indicating that 'small' and 'large' mussels were clearly separated from one another in both the initial and final length distributions for each site. We calculated, for each site, the mean initial and final lengths for 'small' and 'large' mussels.

To calculate our growth rate metric (mm d^{-1}), we subtracted the mean initial length from the mean final length at each site, and divided this value by the respective deployment period (143 days in 2012 and 99 days in 2013). Although site-specific initial lengths were only available in 2013 (when all deployed mussels were measured), a subset of initial lengths (n = 161 mussels) was available for 2012 (Figure 3). From this subset, we randomly generated, with replacement, six samples of 18 mussels each (which matched field deployments in 2012) and used the mean length of those six samples as the 2012 site-specific initial lengths. Growth rates were calculated separately for small and large mussels (2013) and silos and cages (both years).

Correlation analyses were conducted to determine if spatial trends in growth were similar between mussels in cages and silos within each year (mainstem only) and also if spatial trends were consistent between years. Pearson correlation was used for these analyses because growth rates were normally distributed. In addition, we used paired t-tests to compare growth rates between silos and cages each year (mainstem sites only).

We performed regression analyses to determine if and how the growth rates of juvenile mussels were related to their contaminant body burdens (trace metals and PAHs). For these regressions, the dependent variable was site-specific growth rate, and the independent variable was site-specific body burdens of individual metals or PAHs. Exploratory analyses indicated the relationships between growth rates and many metals, and some PAHs, were curvilinear. Indeed, curvilinear (logarithmic) models provided a better fit to the observed data than linear models in the majority (61%) of all regressions. In cases where linear models performed better, the gain in r^2 values was minimal (mean increase = 0.02) and there were no differences in significance relative to the curvilinear models. However, when curvilinear models fit better than linear models, the increase in r^2 was slightly higher (mean increase = 0.05) and changed the designation of significance in ten cases (five metals and five PAHs). Therefore, we based our inferences on regression parameters from curvilinear models, with the exception of Mn, which showed a unique (quadratic) relationship with growth rate (see results).

Spatial variability among sites: The Scheirer-Ray-Hare (SRH) extension of the Kruskal-Wallis test, a two-way non-parametric analog of ANOVA (Sokal and Rohlf 1995) was used to determine if tissue concentrations of individual contaminants (metals or PAHs) differed significantly between deployment methods (cages vs. silos), controlling for site (i.e., an interaction factor was included for site by deployment method). The non-normality and heteroscedasticity of tissue concentration data necessitated a non-parametric test. These SRH tests indicated no significant differences in any PAHs between silos and cages in either year (p-values > 0.3). Some metals (one in 2012, but 8 of 21 in 2013) exhibited significantly different concentrations between cages and silos. Nevertheless, there were no significant interaction terms for these metals; more importantly, their spatial trends were very similar between deployment

methods. Therefore, we assumed combining cage and silo tissue concentration data (by site) would not bias our inferences on spatial trends of tissue contaminant concentrations and used these pooled data for all subsequent analyses.

We performed non-parametric multiple comparisons to test for differences in juvenile mussel tissue concentrations of individual contaminants (metals and PAHs) among sites. Specifically, we used Dunn's test to conduct all possible pairwise comparisons of ranked concentrations of given contaminants among sites and based our inferences on Bonferroni-adjusted p-values (Zar 1999). Multiple comparisons were performed in JMP Pro (version 12.0.1, SAS Institute, Cary, NC, USA) using an overall (experiment-wise) alpha of 0.05.

We performed cluster analyses on juvenile mussel tissue concentrations (separately for metals and PAHs) to determine if there were groups of sites with similar contaminant signatures and whether any contaminants co-varied similarly among sites. Specifically, we conducted hierarchical agglomerative cluster analyses using the average group linkage method and Euclidean distances in Statistical Analysis Software (SAS, version 9.3, SAS Institute, Cary, NC, USA). We excluded analytes that were below detection limits (BDL) among all sites for a given analysis. However, for analytes that were BDL for some, but not all sites, we substituted half the detection limit for their concentrations. Due to the relatively large variation of concentrations among analytes measured, it was necessary to standardize data (within a constituent) to ensure that analytes with high concentrations were not altering the results (e.g., Si concentrations in juvenile tissues ranged from 108 to 2219 ng/g while Cd was orders of magnitude less and ranged from 0.3 to 1.9 ng/g). To determine the optimal number of clusters (groups) in each analysis, we assessed multiple diagnostics, including the pseudo F and pseudo t^2 statistics (generated by SAS), and the relationship between the percent of total variation explained versus

the number of clusters (e.g., this would range between 1 and 12 potential clusters because there were 12 sites). These diagnostics generally provided a consensus on the optimal number of clusters because the local maxima of pseudo *F* often coincided with the point (number of clusters) where pseudo t^2 sharply increased at the next cluster fusion and beyond, at which the amount of variability (information) explained sharply declined, as the number of clusters decreased (i.e., the inflection point). We used PC-ORD (version 6.16, MjM Software Design, Gleneden Beach, OR, USA) to generate two-way dendrograms for each cluster analysis.

Correlations between all possible pairs of contaminants: We performed correlation analyses to determine the strength of association between all possible pairs of contaminants in juvenile mussel tissues (separately and by year for metals and PAHs). To ensure sufficient sample sizes for these analyses, we only included contaminants that were above detection limits at more than half the sample sites for a given analysis. Furthermore, because a large majority of the data violated the assumption of bivariate normality (required by Pearson correlation), we instead used Spearman correlation, which provides slightly more conservative inferences, but is appropriate for non-normal data such as these. Accordingly, we report Spearman's rho correlation coefficients (r_s), which indicate the magnitude and direction of monotonicity between the two variables of interest. For example, positive values of r_s above 0.6 would indicate a strong positive (monotonic) relationship between the concentrations of two given contaminants (e.g., Al and Pb) in mussel tissues in a particular year. We conducted correlation analyses in SAS and used an alpha value of 0.05 to evaluate whether correlation coefficients significantly differed from 0.

Correlations between juvenile tissue and environmental concentrations: We also conducted Spearman correlation to determine whether juvenile tissue concentrations of analytes were

related to those of their immediate environment (i.e., metal and PAH concentrations measured in the sediment, pore water, and surface water at the same sites where mussels were deployed; see Chapter 2 for environmental concentrations). In addition to conducting mussel tissue by sediment correlations on a year-specific basis, we also correlated analyte concentrations of 2013 juvenile mussel tissue to sediment concentrations in 2012 because we assumed that bed chemistry (measured in 2012) would remain relatively stable between years. Correlations of tissue metal concentrations with surface water concentrations were conducted by sampling quarter because, with the exception of Mg, metal concentrations were not significantly correlated among water sampling events Q2, Q3, and Q4 (quarters when metals were measured at all 12 sites including the tributaries). Likewise, correlations of tissue PAH concentrations with surface water (PSDs) were performed by deployment period, namely to determine if tissue concentrations were more tightly coupled with recent PAH exposure (i.e., water concentrations of PAHs measured during deployment periods closer in time to the collection date of mussels).

Correlations between life stages and years: To determine if deployed juvenile mussels exhibited similar spatial trends in accumulation of given contaminants as resident mussels collected from the same sites in a companion study (see Chapter 2), we correlated within each year, via Spearman correlation, the site-specific tissue contaminant concentrations of juveniles with those of resident mussels. In addition, to test for interannual stability in spatial trends of contaminants, we correlated site-specific juvenile tissue concentrations between years 2012 and 2013.

Correlations between density and deployed mussel contaminant concentrations: To determine if there was an association between native mussel density in the Clinch River and body burdens of metals and PAHs from our deployed mussels, we correlated available mussel density data with measured tissue concentrations of juvenile mussels. Mussel densities, based on quantitative

quadrat sampling (number per m^2), were available for all eight of the mainstem sites from which we deployed juvenile mussels in 2012 and 2013 (Krstolic et al. 2013; Jones et al. 2014; J. Jones personal communication). Over the last 10 years (2005-2014), there have been two to three surveys conducted at the mainstem sites, with the exceptions of CAR, CHP, and HF (1 survey only) and WB (sampled every year). We calculated the mean mussel density at each site and correlated this with site-specific tissue concentrations of deployed juvenile mussels within each year (2012 and 2013). As above, we used Spearman correlation and only included in analyses those metals and PAHs that were above detection limits at more than half the sites.

Stable isotopes data analysis: We used paired t-tests to compare $\delta^{15}N$ and $\delta^{13}C$ values between mussels deployed in cages and silos at the same site. These analyses were restricted to the eight mainstem sites because cages were not deployed in the tributaries. Likewise, paired t-tests were conducted to test for differences in $\delta^{15}N$ and $\delta^{13}C$ between juveniles and resident mussels deployed and collected (see Chapter 2) at the same mainstem sites, respectively. Lastly, two-sample t-tests were used to compare tissue values of $\delta^{15}N$ and $\delta^{13}C$ between tributary and mainstem habitats using silo mussels only. All tests were performed separately by year and isotope.

Results

Recovery and Survival

Recovery rates were high overall and similar between cages and silos. In 2012, site-specific recovery rates ranged from 76-99% in cages and 95-100% in silos. In 2013, recovery rates ranged from 97-100% among sites for both silos and cages.

With one exception (caged mussels at CAR), survival was high overall (80-90% in 2012 and near 100% in 2013) and exhibited little spatial variability. In 2012, there were no significant

differences among sites for either caged mussels ($p = 0.10$, ANOVA) or those in silos ($p = 0.41$, ANOVA). However, survival was significantly lower in cages than silos ($p = 0.04$) (Figure 4A). In 2013, survival was similar among sites for mussels in silos ($p = 0.62$, ANOVA), but there was a significant difference among sites for mussels in cages ($p = 0.0001$, Kruskal-Wallis test), again driven by the lower survival at CAR (80%) compared to other sites (100% survival) (Figure 4B). As in 2012, survival was significantly lower in cages than silos in 2013 ($p = 0.05$, paired t-test).

Growth

Although 2013 growth rates were higher overall in small (mean = 0.03 mm d^{-1}) vs. large (mean = 0.01 mm d^{-1}) mussels, both size groups exhibited similar spatial trends in growth among sites as indicated by the strong positive and significant correlation between site-specific growth rates of small and large mussels in both silos ($r_s = 0.91$, $p < 0.0001$) and cages ($r_s = 0.87$, $p = 0.005$). Therefore, we considered it most prudent to use the average growth rate between these two size groups henceforth because a single growth rate (for 2013) facilitated comparisons to 2012 data.

Juvenile mussels grew faster in cages than silos in both years. In 2012, growth rates were higher in cages than silos at all sites (Figure 5A) and the mean difference (0.02 mm d^{-1} higher in cages) was highly significant ($p = 0.006$, paired t-test). In 2013, juveniles grew faster in cages than silos at all but one site (ART) (Figure 5B), and the mean difference (0.01 mm d^{-1} higher in cages vs. silos) was also significant ($p = 0.02$, paired t-test).

Deployed mussels exhibited spatial variation in growth rates, with some consistencies in growth rate trends between years. First, growth was generally higher at WB and HF than other sites, especially for caged mussels (Figure 5). Growth rates of caged mussels at these two sites exceeded 0.03 mm d^{-1} in both years, and the highest growth rates in the study of nearly 0.05 mm

d^{-1} occurred at WB. Secondly, growth in tributaries (except CC) was consistently higher than in mainstem sites during both years for mussels in silos (Figure 5). Nevertheless, spatial trends in growth in silos did not track cage growth particularly well as evidenced by the moderate (r_s values 0.53 – 0.55), but non-significant correlations (p-values 0.16 – 0.18) between mussel growth in cages and silos within each year (Table 1). Nor was there good agreement in spatial trends of cage growth between years (r_s = 0.38, p = 0.36). The agreement of spatial growth trends between years was stronger for mussels deployed in silos (r_s = 0.79, p = 0.02), especially when tributary sites were added to the analysis (r_s = 0.84, p = 0.0007).

Tissue contaminant concentrations

Metals: Tissue concentrations of metals were generally higher and more variable in 2012 than 2013. Specifically, 15 of 21 metals had a greater range of tissue concentrations in 2012 than 2013, and for 10 of those metals, the range was three to 10-fold higher in 2012. Numerous metals in 2012 (Al, Co, Fe, Pb, Si, and V) exhibited a similar spatial trend whereby concentrations decreased downriver with the exception of a spike at CC (Figure 6). The similarity in spatial trends of these metals is also illustrated by their strong positive pairwise correlations (r_s values from 0.78 – 0.99, Table 2) and the fact they formed a distinct cluster that differentiated the most upstream (ART, CLE, CAR) vs. downstream (SIM, PEN, CLP, HF, WB) sites in the mainstem (Figure 7). Chromium concentrations also exhibited a downstream decrease, but peaked at GR instead of CC (Figure 6). Each of these individual metals had significantly higher concentrations at one or both of the upstream sites (CAR, CLE) compared to the most downstream sites (HF, WB) (Table 3).

The tributary sites, namely DC and GR, exhibited unique metal tissue concentrations. In both years, DC formed a distinct cluster from other sites (Figures 7, 8) with relatively high

concentrations of As, Ba, Mn, and Sr and low concentrations of Sb and Hg each year (Figure 6). In 2012, GR formed a distinct cluster with a metal signature of high Cr, Cu, and Ni and low Cd, K, Se, and Zn (Figure 7).

Organics: In 2012, concentrations of total polychlorinated biphenyls (PCBs) were generally less than 1 ng/g (dry weight) at all sites indicating very low accumulation of PCBs in mussels. However, the highest concentrations (0.8 – 1.0 ng/g) were found at the upstream sites (ART, CLE, and CAR), while all other sites had concentrations < 0.3 ng/g. Interestingly, concentrations of PCBs in juvenile mussels were nearly identical to concentrations in resident mussels at each site (see Chapter 2). The PCBs detected were primarily those in the 5-6 Cl range (101, 118, 153, 105, and 138) as individual PCB congener concentrations were below 1 ng/g. No PCBs were detected in any samples in 2013.

Regarding pesticides, in 2012, mussels at only one site (CC) contained detectable amount of any organochlorine pesticides, and these consisted of very low concentrations of 4,4'-DDE and 2,4'-DDD, with all detections well below 1 ng/g. None were detected in 2013. In neither year were current-use pesticides detected in mussel samples.

Unlike other organic compounds, PAHs were prevalent in mussel tissues. The salient spatial trend in PAH tissue concentrations was the strong peak at GR in both years. In 2012, total PAH (the sum of all 42 individual PAHs analyzed) tissue concentrations exceeded 5,000 ng/g at GR, with the next highest levels observed just downstream of GR at SIM (941 ng/g) and PEN (582 ng/g) (Figure 9). Tissue concentrations at the other sites were much lower and ranged from 41 to 386 ng/g, with a mean of 223 ng/g (Figure 9). In 2013, a similar spatial trend was observed whereby total PAH concentrations were much higher at GR than other sites, with a secondary peak at DC (Figure 9).

The suite of individual PAHs that contributed to the unique signature of the GR differed between years. In 2012, six PAHs (C2, D1, D3, FP2, P3, and P4) were only detected in juveniles deployed at the GR; these PAHs comprised a distinct cluster in the 2012 dendrogram (Figure 10). However, in 2013, a year in which fewer PAHs were detected (16 vs. 28 in 2012; Table 4), none of the six PAHs that constituted the 2012 signature of the GR were detected in juvenile mussels. Instead, the following PAHs exhibited relatively high concentrations that contributed to the 2013 GR signature and were also high at DC: C1, F1, FP1, N1, N2, N3, and P2 (Figure 11 dendrogram; Table 5).

Stable Isotopes

Isotope values did not differ between mussels deployed in cages and silos at the same site, regardless of isotope or year, as p-values ranged from 0.12 to 1.0 across all paired t-tests (also see Figure 12). Due to this similarity of isotopic signatures between cages and silos, we pooled data for all subsequent analyses (i.e., across deployment methods for juveniles in the mainstem).

There were strong differences in isotopic signatures among life stages as indicated by the highly significant differences (all p-values <0.0001) in the paired t-tests. Specifically, $\delta^{15}N$ was enriched in residents compared to juveniles, and $\delta^{13}C$ exhibited higher values in residents than juveniles, each year (Figure 12). In addition, the isotopic signatures of juveniles and residents were clearly distinguishable, substantially enriched (^{15}N) or higher (^{13}C), than the baseline mussels from the hatchery (Figure 12).

Although fewer in number (n=4), tributary sites exhibited much greater variability in isotope values than mainstem sites (n=8). Nitrogen was generally more depleted in tributaries, with the exception of GR, which exhibited the highest $\delta^{15}N$ values of all sites in both 2012 (7.5‰) and 2013 (8.5‰). When GR was excluded from the analysis, the t-test indicated that

$\delta^{15}N$ was more depleted in the tributaries than the mainstem in each year (2012: p=0.004; 2013: p=0.003). Within the tributaries, $\delta^{13}C$ was lowest at DC (-30.3‰) and highest at GR (-27.3 to -26.5‰) across years. Values of $\delta^{13}C$ of the two other tributary sites were generally similar to mainstem sites (~ -27‰), however, this low sample size (n=2) precluded statistical inference of $\delta^{13}C$ comparisons between habitats. (Figure 12)

Relationships of contaminants between tissues and the environment

Metals

Pore water: Concentrations of metals in juvenile mussel tissues was unrelated to the spatial pattern of variability of metals in pore water, as indicated by the general lack of significant correlations between pore water and mussel tissue concentrations (Table 6). In general, r_s values among metals were below 0.4 with a few exceptions (Figure 13). Magnesium concentrations in juveniles exhibited a significant positive relationship with pore water concentrations in both June (r_s = 0.68, p = 0.02) and October (r_s = 0.66, p = 0.02). In addition, Zn concentrations in mussel tissues showed a marginally significant positive association with pore water concentrations in October (r_s = 0.50, p = 0.10), but not June (r_s = -0.36, p = 0.26).

Sediment: Tissue concentrations of metals in mussels showed little relation with metal concentrations in the surrounding sediment (Table 7). Most r_s values (77%) for all tissue by sediment correlations were below 0.3 (Figure 13). However, there were a few exceptions including a significant positive correlation between juvenile As and particulate sediment (r_s = 0.74, p = 0.01); marginally significant positive correlations of juvenile Al (r_s = 0.53, p = 0.08) and Cr (r_s = 0.54, p = 0.08) with bed sediment and marginally significant positive relationship between Zn and particulate sediment (r_s = 0.62, p = 0.07). Marginally significant negative correlations existed between Ni in juveniles and both bed (r_s = -0.52, p = 0.08) and particulate

sediments (r_s = -0.58, p = 0.06); a significant negative relationship between K and bed sediment (r_s = -0.71, p = 0.02), and a marginally significant negative relationship between Sb and particulate sediment (r_s = -0.65, p = 0.06).

Surface water: While the majority (76%) of correlations between metal concentrations in mussel tissues and those in the water column were not significant, the water column was the environment in which we observed the strongest positive correlations with mussel tissue concentrations as compared to pore water and sediment (Figure 13). Specifically, 2013 juveniles exhibited strong positive (r_s values > 0.71) and significant relationships (p-values < 0.04) with 5 of the 8 metals (Al, Fe, K, Mg, and Mn) that were above detection limits (among sites) during Q5 (Table 8). Juvenile Mn and Mg also showed significant positive correlations with water column concentrations during Q2 (r_s = 0.68, p = 0.02) and Q4 (r_s = 0.58, p = 0.05), respectively.

PAHs

Pore water: Juvenile tissue concentrations showed little association with surrounding pore water PAH concentrations. Tissue concentrations only exhibited a significant positive relationship with 1 of 13 PAHs analyzed (P1 in the June 2013 pore water sample) (Table 9). Tissue concentrations showed a significant positive relationship (r_s = 0.69) with total PAH concentrations measured in the October 2013 pore water sample, but not the June 2013 sample (Table 9).

Sediment: Juvenile tissue concentrations generally exhibited weak to moderate positive correlations with sediment PAH concentrations (Figure 13), with a few strong correlations observed in 2013 juveniles (Table 10). In 2012, tissue concentrations showed a significant positive relationship with N3 and marginally significant relationships with N4 and F1. Juvenile tissue concentrations in 2012 were weakly, but positively correlated with total PAH

concentrations. Tissue concentrations of juveniles deployed in 2013 showed slightly higher correlations to particulate sediment PAH concentrations (2013) than bed sediment (2012) concentrations for 7 of 10 PAHs (Table 10). Significant positive correlations were observed between tissue and particulate sediment concentrations of F, FP1, N, P, P1, and PY. Total PAH concentrations in tissues demonstrated a significant positive relationship with both bed (2012) and particulate (2013) sediment concentrations (Table 10).

Surface water: Tissue concentrations of PAHs exhibited much stronger (positive) relationships with surface waters compared to other environmental compartments (pore water and sediment; Figure 13). In 2012, the relationships between tissue and surface water concentrations were at least marginally significant for all PAHs except N1 (Table 11). Particularly strong correlations were observed between tissue and PAHs in surface waters collected during the fall 2012 PSD deployment period. For instance, r_s was 0.98 (near perfect agreement) between total PAH tissue and surface water concentrations (Figure 14). A lower percentage of 2013 relationships were significant (21% compared to 81% in 2012). Summer 2013 was the deployment period with the highest percentage (4 of 11) of significant positive relationships. The PAH N3 showed a significant positive relationship in all deployment periods in 2013. Total PAH concentrations were positively, but not significantly, correlated with surface waters across the 2013 deployment periods.

Tissue correlations among years and life stages

There was greater interannual stability between years in the spatial trends of PAHs than metals in juvenile mussels. Correlations between years were negative for 11 of 18 metals, and only 2 metals showed at least a moderate positive correlation between years (Mn: $r_s = 0.69$, p = 0.01; Sr: $r_s = 0.5$, p = 0.1) (Table 12). Meanwhile, all 10 PAHs except N1 exhibited a positive

correlation between years, 4 of which were significant (AN, C, FL, and N3) and one marginally significant (PY), in addition sum42 (Table 13).

Spatial trends in concentrations of individual PAHs were highly correlated between life stages (juveniles and resident mussels) within each year (2012 and 2013). In 2012, 16 of the 18 PAHs exhibited a strong positive and significant relationship between life stages; in 2013, all 11 PAHs were significantly and positively correlated between life stages (Table 14). Correlation coefficients for individual PAHs between life stages within a year ranged from 0.72 – 1.0, with a mean of 0.89; very strong correlations were also observed for total PAH concentrations (r_s = 0.85 in 2012 and 0.96 in 2013) (Table 14).

In contrast to PAHs, only some metals were highly correlated between life stages within a year. In 2012, the following metals exhibited a significant positive relationship between years: Al, Cr, K, Mo, Pb, Si, and V (Table 15). In 2013, none of the 20 metals that were consistently above detection limits were significantly correlated. Interestingly, those metals that showed a significant relationship between life stages in 2012 were not significantly correlated between years in resident mussels. Meanwhile, those metals whose spatial trends were significantly correlated between years in resident mussels (As, Ba, Fe, Hg, Mg, Mn, Sr, and Zn) were not correlated between life stages within a year (Table 15). This finding suggests some metals (e.g., Al, Pb, Si, V) measured in resident mussel tissues may be more reflective of recent exposure (< 1 year) than others (e.g., As, Ba, Hg, Mg, Mn, and Sr).

Relationships between growth rate and contaminant concentrations

Significant relationships between growth rate and tissue concentrations of trace metals were observed only for caged mussels (especially in 2012). Moderately strong negative relationships (r^2 values of 0.53 – 0.68) between growth rate and tissue concentrations were

observed for 9 trace metals in 2012 caged mussels, including Al, Co, Cr, Cu, Fe, K, Ni, Si, and V. In addition, marginally significant negative relationships ($p = 0.06$) were observed for Mo and Ni. The negative curvilinear pattern for these 11 metals indicates that growth rates were depressed at high tissue concentrations, but increased rapidly below an apparent threshold value, which differed among metals. For instance, growth rapidly increased at Cu concentrations below 90 µg/g and Ni concentrations below 2.5 µg/g (Figure 15). Interestingly, growth rate exhibited a quadratic relationship with Mn in 2012 caged mussels, whereby growth increased from 1850 to 2300 µg/g but decreased at higher concentrations (up to 2800 µg/g) (Figure 16). In 2013 caged mussels, there were few significant relationships observed, as growth rates exhibited a significant negative relationship with Ba and significant positive relationship with Mo.

In contrast to metals, the growth rates of juvenile mussels showed little relation to PAH body burdens. The growth rates of caged mussels in 2012 showed a significant negative relationship (r^2 of 0.56 – 0.57, p-values of 0.03) with only 3 of 20 PAHs (BaA, F1, and PER). Furthermore, there were no significant relationships between growth rates and PAH tissue concentrations for mussels deployed in silos in 2012 or cages in 2013. Growth rates of mussels in silos in 2013 exhibited a significant positive relationship with C and total PAH concentration, however these inferences must be interpreted with caution because the fit of the relationships were moderate at best (r^2 from 0.39 – 0.41).

Correlations between native mussel density and juvenile mussel contaminant concentrations

Mussel densities showed little association with metal tissue concentrations. There were no significant correlations between mussel density and the site-specific tissue concentrations of any metals in either year, with the exception of K and Sb in 2012 (Table 16). However, the

correlation with these metals is likely spurious due to the limited range of values for both measured in 2012 (3232 – 3977 µg/g for K, and 0.50 – 0.68 µg/g for Sb) compared to 2013 (1858 – 2889 µg/g for K, and 0.28 – 0.52 µg/g for Sb), when no significant relationships were observed (r_s = 0.12, p = 0.78 and r_s = -0.19, p = 0.65, respectively).

In contrast to metals, mussel densities exhibited a strong negative relationship with tissue PAH concentrations. Twenty of the 42 PAHs were above detection limits for at least one of the sampling years. Total PAH in 2012 had a moderately negative relationship with mussel density (r_s = -0.48), but the correlation was not statistically significant (p = 0.23). Thirteen of the 19 individual PAHs detected in 2012 showed a moderate to strong negative relationship with site-specific mussel density as r_s values ranged from -0.40 to -0.93, though only four were at least marginally significant ($p \leq 0.10$; Table 17).

Mussel densities were strongly correlated with 2013 Total PAH in juvenile tissue (r_s = -0.90, $p < 0.01$), and six of the 10 individual PAHs evaluated for that year had at least a marginally significant relationship with site-specific mussel density ($p \leq 0.10$; Table 17). The trend displayed in Figure 17, whereby the highest mussel densities clearly occurred at the lowest total PAH concentrations, followed by declining densities thereafter as tissue concentrations increased, was representative of the pattern displayed by the individual PAHs that exhibited a negative relationship with mussel density.

Discussion

Our study demonstrated that contaminants had sub-lethal effects on juvenile mussels that manifested themselves in growth but not necessarily in survival, at least over the duration of our field deployments (5 – 6 months) in the Clinch River. The identification of the specific contaminants and their concentrations that caused reduced growth rates will aid future

management and conservation efforts by guiding any mitigation efforts to curtail major inputs of these metals and PAHs to the system. In addition, our study provides valuable insight for mussel field investigations, as we compare the relative merits of two different deployment methods (cages vs. silos), illustrate the importance of multi-year studies, and discuss our results in the context of biomonitoring tools for imperiled mussel populations.

Contaminants in the Clinch River were not acutely toxic to *V. iris* and *L. fasciola* as indicated by the high survival among sites (80-100%) in both years. One exception to this trend was for caged mussels deployed at CAR, where survival rates were 52% in 2012 and 82% in 2013. This site is situated just downstream of a coal-fired power plant (Carbo, VA). Hence, the sediment and/or pore water at CAR may be toxic to juvenile mussels. Interestingly, the survival of mussels in silos, which were primarily exposed to contaminants in the water column and not the sediment, was much higher at this same site in both 2012 (90%) and 2013 (100%). Based on our monthly site visits, this site was also more prone to sediment deposition than other sites, which also could have contributed to the lower survival of caged mussels, though there were no cages with 100% mortality at the site.

The magnitude of survival estimates obtained from *in situ* mussel studies depends heavily on the length of the deployment period. Although survival was high overall in our study, it was clearly lower in 2012 (52 – 96% for caged mussels; mean = 82%) than 2013 (82 – 100%, mean = 98%), when mussels were deployed for a longer period of time (by 1.5 months). This interannual difference in survival could be due to the use of different species in each year. However, in a companion study, Rogers (2015) found much lower survival (27 – 86%, mean = 54%) for the same species (*V. iris*) deployed in cages on the same date and locations (eight mainstem sites) as our juveniles, but for nearly an entire year (344 days) from June 2012 through

May 2013, with no cage maintenance during winter due to high water levels. Therefore, a more plausible explanation for the interannual difference in survival that we observed is that mussels deployed for a longer period (2012) experienced higher cumulative mortality as they were exposed to multiple stressors for a longer duration of time. Interestingly, Rogers (2015) also found significantly higher survival at sites upstream and downstream of the ZOD (mean survival = 65%) compared to sites within the ZOD (mean survival = 44%). We did not observe this strong spatial trend in survival, but this was likely due to our shorter deployment period.

PAHs emerged as the organic contaminants of concern in the Clinch River, and we found extremely high concentrations of PAHs in juvenile mussels deployed at tributary sites (GR and DC) where no extant mussel populations occur. As with adults (see Chapter 2), site-specific mussel densities (lower in the ZOD) exhibited a significant negative relationship with juvenile tissue concentrations of PAHs. Collectively, these results suggest that PAHs may exert a chronic and eventually lethal effect on mussels that has, and could continue to, negatively impact remaining mussel populations within the ZOD. Given the tight coupling of tissue concentrations (of both juveniles and adults) with local environmental concentrations of PAHs (particularly in the sediment and water column), the major PAH sources within our study reach appear to be the mining-associated tributaries of GR and DC.

Our study clearly demonstrated that high body burdens of certain metals depressed the growth of juvenile mussels. In particular, the growth rates of caged mussels were negatively related to tissue concentrations of a number of metals (Al, Co, Cr, Cu, Fe, K, Ni, Si, and V) in 2012, but not in 2013. The nature of this negative relationship (curvilinear) suggested that while growth was depressed at high tissue concentrations, growth rates rapidly increased when body burdens were reduced below a given threshold (e.g., < 90 µg/g for Cu). Many of the metals that

had a negative effect on growth are associated with specific mining activities and known to be toxic to mussels (Silva et al. 2011; Wang et al. 2007, 2013; Besser et al. 2015); indeed, these metals behaved similarly, as evidenced by the high correlations among metals and the fact they clustered together. When growth rate is superimposed on the percent contribution from each metal cluster to the total metal load based upon standardized tissue concentrations (Figure 18), we observed higher concentrations of these metals (Al, Co, Cr, Cu, Fe, Ni, Si, and V) upstream where there is low to intermediate growth versus downstream of the ZOD (WB and HF), where the highest growth rates occurred. The concentrations of these metals were considerably lower in both mussel tissues and the environment in 2013 versus 2012 (see Chapter 2 also), which likely explains the lack of a relationship between growth and metal concentrations in 2013 (i.e., tissue concentrations were too low to elicit negative growth effects). Other field studies have qualitatively noted lower growth of deployed mussels within the ZOD (Johnson et al. 2014; Rogers 2015), but did not statistically model growth rates as a function of tissue concentrations. Lastly, growth rate exhibited a unique quadratic relationship with Mn, which is not surprising given that Mn is an essential micronutrient used in shell-building (Siegele et al. 2001); there likely exists optimal concentrations of Mn that, when exceeded, may be detrimental to mussel health (see Chapter 4). Interestingly, we did not observe the same relationship with growth rate in the silos, however, other constraints on mussels in the silos, which may not support optimal growth, most likely masked any effects of contaminants.

The spatial trends in growth rates that we observed (higher growth downstream of the ZOD) do not appear to be an artifact of differences in water temperature along the mainstem. Based on available gage data from the United States Geological Survey (http://waterdata.usgs.gov/nwis), the median absolute daily difference in water temperature

between CLE (gage 03524000, rkm 436) and SIM (gage 03524740, rkm 378) during the 2012 deployment period was just 0.2^{o}C (these were the only 2 sites with available data for the locations and time periods of interest). Despite these similar temperature histories, the growth rate of caged mussels at SIM was nearly twice that of mussels at CLE, suggesting that water temperature was not the primary driver of the growth differences we observed within the mainstem. However, temperature may explain the generally higher growth rates of silo-deployed mussels in the tributaries (except CC) compared to mainstem sites. Although continuous temperature data were not available from the tributaries, these sites were typically shallower, and presumably warmer, than mainstem sites. Thus, the mussels that survive in the tributaries, in the face of other water quality stressors, may experience faster growth due to warmer water temperatures, or possibly other factors such as increased nutrient availability. This is exemplified by the stable isotope data for mussels in the silos at the tributary sites, which indicate different sources of nitrogen and potentially carbon (at DC) than in the mainstem of the river. The relatively low growth at CC in both years compared to other tributaries may be attributed to the high levels of ammonia in the particulate sediment of CC, which was found to be toxic to young juvenile mussels (1.9 mm mean initial length) in a 28-day laboratory test (see Chapter 4).

As one of the first field investigations using cages for *in situ* mussel deployments, our study provided valuable insight into the advantages and disadvantages of cages relative to the more conventional silo approach. We hypothesized that mussels would accumulate contaminants differently in the cages and silos because they may be feeding differently in a benthic setting versus confinement to the water column. However, this hypothesis was not supported by the stable isotope data which showed mussels deployed in the mainstem in cages

and silos were feeding on similar nitrogen and carbon sources, and we found little differences in metal or PAH accumulation between the two deployment methods. In contrast, growth rates were considerably higher (often double) in cages versus silos, likely because cages better approximate the natural habitat of mussels by providing access to both the sediment and water column above (resulting in increased food supply) compared to silos which are more isolated from the sediment. This constraint on food resources could explain, in part, the lower growth rates of mussels in silos than cages, especially given that tissue concentrations of contaminants were essentially the same between these two deployment methods. Nevertheless, the increased biological realism afforded by cages comes at the costs of higher maintenance and labor. For example, the screens of cages need to be routinely cleaned to prevent the suffocation of mussels due to sediment deposition; the frequency of such cleanings depends on the depositional properties of the study system. In our study, recovery and survival in the silos was near 100% at most sites, but slightly lower in cages, which could have been due, in part, to sediment deposition. Also, silos may be the only deployment option in hard substrates, as minor bed excavation is required to deploy cages. Accordingly, the deployment method largely depends on the study questions and physical properties of the study area.

Deployed mussels had a clear and detectable shift in isotopic signatures from the baseline (hatchery) mussels during the 5 – 6 month deployment period and clustered more closely with the signatures of resident mussels in the mainstem. Thus, not surprisingly, we observed differences in metal and PAH uptake in juveniles between years, similar to resident mussels (Chapter 2), that highlight the dynamic nature of this watershed. Tissue concentrations of metals were generally higher in 2012 than 2013, but for PAHs, the converse was true. Moreover, the suite of constituent PAHs (i.e., individual PAHs comprising the total PAH load) differed greatly

between years as some constituent PAHs were only observed in one year or the other. These findings illustrate the importance of conducting multi-year ecological studies in dynamic fluvial systems. For instance, had we only sampled in 2013, when flows were generally higher during the growing season compared to 2012, we would have missed the high concentrations of metals that accumulated in mussel tissues and caused reduced growth.

Given the dynamic nature of contaminant concentrations, one might expect juveniles to better track contaminant accumulation in a given growing season or year than adults (residents), which have been exposed to contamination for numerous years. However, we found that tissue concentrations of contaminants (both metals and PAHs) exhibited much stronger (positive) correlations between life stages (juvenile and residents) within a given year than between years within a given life stage. This finding suggests that tissue concentrations of adults reflect recent contaminant exposure (i.e., < 1 year). At least for PAHs (not metals), tissue concentrations of both juveniles and adults (see Chapter 2) were tightly coupled with their environmental concentrations, especially the more time-integrative PSD samples of surface water PAHs. Accordingly, PSD sampling could serve as a proxy for PAH tissue loads in live mussels and constitute an important biomonitoring tool for imperiled species that should not be sacrificed.

In conclusion, our multi-year field study (this chapter and Chapter 2) provided new data on the potential role of contaminants in freshwater mussel declines within the Clinch River. Our study identified PAHs as the major organic contaminant of concern in this system. Our results demonstrated a clear link between PAH body burdens and mussel health given the strong negative correlations between site-specific mussel density and PAH tissue concentrations measured in both juveniles and adults. While PAHs appear to have a potentially chronic lethal effect on mussel populations in the Clinch River, metals seem to exert more subtle sub-lethal

effects on growth, at least in years when metals are able to reach high enough concentrations in mussels to elicit negative effects. Our identification of the class of organic contaminants and individual metals of concern will aid management and conservation efforts by guiding future studies of the likely sources of those specific contaminants and assist in identifying contaminants to focus on for laboratory toxicity studies. However, it is important to bear in mind that contaminants are not the sole stressor on mussels in this system, as they undoubtedly interact with other environmental factors such as conductivity and turbidity (Johnson et al. 2014) in a complex manner that may result in non-additive and unpredictable effects on mussel health and survival.

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Table 1. Correlation matrix for mussel growth rates within and between years and deployment methods (cages, silos). Pearson correlation coefficients (*r*) are the top values in each cell and associated p-values are italicized below *r* values. Only mainstem sites (n = 8) were included in analyses because only at those sites were mussels were deployed in both cages and silos.

	Silos 2012	**Cages 2013**	**Silos 2013**
Cages 2012	0.55 *0.16*	0.36 *0.38*	0.04 *0.93*
Silos 2012		0.56 *0.15*	0.79 *0.02*
Cages 2013			0.53 *0.18*

Table 2: Matrix reporting Spearman's rho correlation coefficients (r_s) among all possible pairs of site-specific mean tissue concentrations of metals in juvenile mussels within each year. The top, off-diagonal, portion of the matrix (above the black line) shows r_s values for 2012 correlations and the bottom, off-diagonal, portion shows r_s values for 2013 correlations. Cells with gray-fill and bolded and italicized text indicate r_s values were significantly different than 0.

2012

	Al	**As**	**Ba**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**K**	**Mg**	**Mn**	**Mo**	**Ni**	**Pb**	**Sb**	**Se**	**Si**	**Sr**	**V**	**Zn**	
Al		0.20	0.35	-0.08	***0.90***	***0.69***	0.53	***0.78***	0.22	***0.61***	***0.78***	-0.24	0.48	0.37	***0.96***	0.38	-0.08	***0.99***	0.30	***0.93***	-0.24	**Al**
As	-0.03		0.21	0.55	0.08	-0.15	0.06	0.13	-0.08	0.17	0.39	0.07	-0.20	-0.15	0.20	0.01	***0.81***	0.16	0.11	0.37	0.43	**As**
Ba	0.11	***0.73***		***0.60***	0.38	0.15	0.17	0.34	-0.04	0.15	0.43	0.47	0.27	0.30	0.43	0.17	0.30	0.33	***0.78***	0.57	***0.62***	**Ba**
Cd	0.31	***0.61***	0.41		-0.14	-0.43	-0.33	-0.29	0.08	-0.19	0.13	0.48	-0.35	-0.33	-0.03	-0.15	***0.59***	-0.15	0.41	0.18	***0.68***	**Cd**
Co	***0.83***	0.15	0.11	0.43		***0.83***	***0.59***	***0.92***	0.06	0.34	***0.76***	0.05	***0.71***	0.57	***0.88***	0.11	-0.08	***0.89***	0.46	***0.80***	-0.29	**Co**
Cr	***0.76***	-0.29	-0.07	0.25	0.52		***0.86***	***0.85***	-0.03	0.19	0.55	-0.07	***0.66***	***0.66***	***0.70***	0.10	-0.22	***0.70***	0.29	0.57	-0.41	**Cr**
Cu	***0.59***	-0.03	0.29	0.32	0.33	0.48		***0.75***	-0.16	0.23	0.38	-0.07	0.46	0.57	***0.61***	0.26	0.11	0.52	0.29	0.50	-0.10	**Cu**
Fe	***0.84***	0.11	0.24	0.29	***0.90***	0.57	0.39		-0.12	0.37	***0.67***	0.03	***0.74***	***0.70***	***0.80***	0.18	0.06	***0.79***	0.48	***0.71***	-0.20	**Fe**
Hg	-0.22	0.08	0.15	-0.05	-0.50	-0.24	0.19	***-0.59***		0.17	-0.15	-0.31	-0.10	-0.14	0.20	0.01	-0.31	0.16	-0.36	0.20	-0.04	**Hg**
K	0.44	0.10	0.08	0.47	***0.73***	0.08	0.15	***0.59***	-0.50		0.20	***-0.70***	0.30	0.35	0.50	***0.87***	-0.03	***0.62***	0.02	0.55	-0.17	**K**
Mg	0.33	0.33	0.13	***0.62***	***0.65***	0.01	0.12	0.48	-0.43	***0.85***		0.17	0.25	0.12	***0.79***	0.01	0.15	***0.80***	0.45	***0.81***	0.02	**Mg**
Mn	***0.74***	0.24	0.43	0.28	***0.78***	0.27	0.56	***0.87***	-0.31	***0.63***	0.49		0.02	-0.04	-0.08	-0.57	0.38	-0.26	0.53	-0.10	0.52	**Mn**
Mo	0.00	-0.51	-0.37	-0.03	0.02	0.30	0.08	0.13	***-0.63***	0.25	0.05	-0.06		***0.93***	0.40	0.17	-0.27	0.48	0.26	0.30	-0.42	**Mo**
Ni	0.48	-0.14	0.40	-0.04	0.34	0.50	0.44	0.38	0.01	0.16	-0.14	0.39	0.08		0.31	0.30	-0.12	0.36	0.31	0.24	-0.29	**Ni**
Pb	***0.90***	-0.06	0.13	0.33	***0.71***	***0.92***	***0.59***	***0.79***	-0.31	0.22	0.15	0.57	0.16	0.50		0.33	0.03	***0.95***	0.42	***0.94***	-0.05	**Pb**
Sb	-0.11	-0.07	-0.11	0.22	0.10	0.31	0.05	0.06	-0.20	-0.11	0.11	-0.19	0.19	-0.06	0.15		0.03	0.40	0.08	0.33	-0.04	**Sb**
Se	0.49	0.48	0.31	0.41	0.57	0.10	0.13	***0.63***	-0.39	0.48	0.29	***0.66***	0.06	0.06	0.38	-0.36		-0.12	0.40	0.14	***0.65***	**Se**
Si	***0.90***	-0.25	0.03	0.20	***0.65***	***0.93***	0.55	***0.75***	-0.26	0.22	0.14	0.52	0.18	0.50	***0.96***	0.12	0.22		0.31	***0.92***	-0.26	**Si**
Sr	0.11	0.39	0.49	0.19	0.21	-0.21	0.10	0.14	-0.02	0.39	***0.58***	0.30	-0.27	0.18	-0.08	-0.25	-0.03	-0.05		0.48	0.40	**Sr**
V	***0.63***	0.38	0.31	***0.70***	***0.83***	0.31	0.31	***0.71***	-0.41	***0.83***	***0.92***	***0.67***	-0.01	0.15	0.49	0.07	0.45	0.45	0.55		0.09	**V**
Zn	***0.66***	***0.62***	***0.62***	***0.77***	***0.68***	0.36	0.45	***0.67***	-0.20	0.55	0.52	***0.71***	-0.05	0.34	***0.59***	-0.13	***0.76***	0.45	0.34	***0.76***		**Zn**
	Al	**As**	**Ba**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**K**	**Mg**	**Mn**	**Mo**	**Ni**	**Pb**	**Sb**	**Se**	**Si**	**Sr**	**V**	**Zn**	

2013

Table 3. Results from Dunn non-parametric multiple comparisons of metal concentrations among sites for juvenile mussels by year, 2012 and 2013. Within a column, sites with different letters indicate significant differences (i.e., where Bonferonni-adjusted p-values ≤ 0.05). Only those metals with significant pairwise differences are included here.

	2012 juveniles								*2013 juveniles*				
Site	Al	Co	Cr	Fe	Pb	Si	V	Site	Al	K	Ni	Pb	Si
IC	ab	ab	ab	ab	ab	ab	ab	IC	a	ab	ab	a	a
ART	ab	ab	ab	ab	ab	ab	ab	ART	ab	ab	ab	ab	ab
CLE	ab	ab	a	ab	a	a	a	CLE	ab	a	ab	ab	ab
DC	ab	ab	ab	ab	ab	ab	ab	DC	ab	ab	ab	ab	ab
CAR	a	a	ab	a	a	ab	ab	CAR	ab	ab	ab	ab	ab
GR	ab	ab	ab	ab	ab	ab	ab	GR	ab	ab	ab	ab	ab
SIM	ab	ab	ab	ab	ab	ab	ab	SIM	ab	ab	ab	ab	ab
PEN	ab	ab	ab	ab	ab	ab	ab	PEN	ab	ab	ab	ab	ab
CLP	ab	ab	ab	ab	ab	ab	ab	CLP	ab	ab	ab	ab	ab
CC	ab	ab	ab	ab	ab	a	ab	CC	ab	ab	a	ab	ab
HF	b	ab	b	b	ab	b	b	HF	b	ab	ab	b	ab
WB	b	b	b	b	b	ab	b	WB	ab	b	b	ab	b

Table 4: Matrix reporting Spearman's rho correlation coefficients (r_s) among all possible pairs of site-specific mean tissue concentrations of PAHs in juvenile mussels within each year. The top, off-diagonal, portion of the matrix (above the black line) shows r_s values for 2012 correlations and the bottom, off-diagonal, portion shows r_s values for 2013 correlations. Cells with gray-fill and bolded and italicized text indicate r_s values were significantly different than 0.

2012

	AN	Ace	BaA	C	C1	C2	D	D1	D2	D3	F	F1	F2	F3	FL	FP1	FP2	N	N1	N2	N3	N4	P	P1	P2	P3	P4	PER	PY	
AN		***0.83***	***0.90***	***0.91***	0.47	0.48	***0.74***	0.48	0.22	0.48	***0.94***	***0.69***	***0.73***	0.48	***0.93***	***0.89***	0.48	***0.92***	***0.74***	0.54	***0.73***	***0.80***	***0.89***	-	***0.92***	0.48	0.48	0.43	***0.94***	**AN**
Ace	-		***0.86***	***0.88***	0.35	0.48	***0.74***	0.48	-0.05	0.48	***0.83***	0.55	0.49	0.29	***0.80***	***0.76***	0.48	***0.85***	***0.66***	0.35	***0.65***	***0.74***	***0.65***	-	***0.84***	0.48	0.48	0.31	***0.85***	**Ace**
BaA	-	-		***0.94***	0.54	0.48	***0.76***	0.48	0.10	0.48	***0.96***	***0.63***	0.56	0.32	***0.96***	***0.98***	0.48	***0.85***	***0.72***	0.54	***0.77***	***0.86***	***0.82***	-	***0.92***	0.48	0.48	0.57	***0.97***	**BaA**
C	***0.82***	-	-		0.41	0.48	***0.74***	0.48	0.19	0.48	***0.97***	***0.66***	***0.61***	0.49	***0.89***	***0.90***	0.48	***0.80***	***0.74***	0.48	***0.75***	***0.83***	***0.76***	-	***0.95***	0.48	0.48	0.53	***0.92***	**C**
C1	0.17	-	-	0.41		***0.74***	***0.69***	***0.74***	***0.59***	***0.74***	0.53	0.12	0.41	0.22	0.47	***0.59***	***0.74***	0.47	0.41	0.28	0.41	0.47	***0.66***	-	0.47	***0.74***	***0.74***	0.15	0.47	**C1**
C2	-	-	-	-	-		0.51	***1.00***	0.54	***1.00***	0.48	0.48	0.48	0.51	0.48	0.48	***1.00***	0.48	0.28	0.51	0.48	0.48	0.48	-	0.48	***1.00***	***1.00***	-0.22	0.48	**C2**
D	-	-	-	-	-	-		0.51	0.30	0.51	***0.78***	0.34	***0.66***	0.36	***0.66***	***0.72***	0.51	***0.65***	***0.62***	0.42	***0.61***	***0.66***	***0.70***	-	***0.68***	0.51	0.51	0.27	***0.71***	**D**
D1	-	-	-	-	-	-	-		0.54	***1.00***	0.48	0.48	0.48	0.51	0.48	0.48	***1.00***	0.48	0.28	0.51	0.48	0.48	0.48	-	0.48	***1.00***	***1.00***	-0.22	0.48	**D1**
D2	-	-	-	-	-	-	-	-		0.54	0.26	0.27	0.55	***0.70***	0.05	0.13	0.54	0.19	-0.08	0.22	0.23	0.21	0.44	-	0.16	0.54	0.54	0.06	0.06	**D2**
D3	-	-	-	-	-	-	-	-	-		0.48	0.48	0.48	0.51	0.48	0.48	***1.00***	0.48	0.28	0.51	0.48	0.48	0.48	-	0.48	***1.00***	***1.00***	-0.22	0.48	**D3**
F	***0.76***	-	-	0.53	0.26	-	-	-	-	-		***0.63***	***0.68***	0.48	***0.92***	***0.94***	0.48	***0.84***	***0.78***	0.52	***0.78***	***0.86***	***0.84***	-	***0.97***	0.48	0.48	***0.59***	***0.93***	**F**
F1	0.10	-	-	0.18	***0.67***	-	-	-	-	-	0.49		***0.75***	***0.75***	***0.69***	0.57	0.48	***0.66***	0.15	***0.81***	***0.65***	***0.63***	0.56	-	0.57	0.48	0.48	0.17	***0.68***	**F1**
F2	-	-	-	-	-	-	-	-	-	-	-	-		***0.84***	0.56	0.53	0.48	***0.64***	0.29	***0.72***	***0.65***	***0.64***	***0.65***	-	0.56	0.48	0.48	0.19	0.56	**F2**
F3	-	-	-	-	-	-	-	-	-	-	-	-	-		0.31	0.27	0.51	0.39	0.06	***0.64***	0.50	0.44	0.40	-	0.38	0.51	0.51	0.05	0.32	**F3**
FL	***0.78***	-	-	***0.64***	-0.09	-	-	-	-	-	0.56	-0.04	-	-		***0.97***	0.48	***0.87***	***0.71***	***0.59***	***0.76***	***0.83***	***0.82***	-	***0.90***	0.48	0.48	0.48	***0.99***	**FL**
FP1	0.19	-	-	0.25	0.49	-	-	-	-	-	0.38	***0.74***	-	-	0.18		0.48	***0.80***	***0.76***	0.53	***0.76***	***0.85***	***0.82***	-	***0.92***	0.48	0.48	***0.59***	***0.96***	**FP1**
FP2	-0.22	-	-	0.04	0.60	-	-	-	-	-	0.13	0.52	-	-	-0.39	0.23		0.48	0.28	0.51	0.48	0.48	0.48	-	0.48	***1.00***	***1.00***	-0.22	0.48	**FP2**
N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0.57	0.44	***0.59***	***0.69***	***0.90***	-	***0.82***	0.48	0.48	0.39	***0.88***	**N**
N1	0.51	-	-	0.51	0.57	-	-	-	-	-	0.69	***0.62***	-	-	0.15	0.35	0.48	-		0.19	0.50	***0.59***	0.54	-	***0.83***	0.28	0.28	0.41	***0.72***	**N1**
N2	0.07	-	-	0.24	***0.64***	-	-	-	-	-	0.36	***0.73***	-	-	-0.20	***0.60***	0.48	-	0.55		0.49	0.46	0.41	-	0.40	0.51	0.51	0.04	0.56	**N2**
N3	0.26	-	-	0.48	***0.64***	-	-	-	-	-	0.51	***0.67***	-	-	0.06	0.56	0.48	-	0.46	***0.88***		***0.98***	0.57	-	***0.78***	0.48	0.48	0.33	***0.75***	**N3**
N4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		***0.66***	-	***0.87***	0.48	0.48	0.47	***0.82***	**N4**
P	***0.72***	-	-	***0.58***	0.52	-	-	-	-	-	***0.92***	0.50	-	-	0.47	0.45	0.31	-	***0.73***	0.38	0.53	-		-	***0.78***	0.48	0.48	0.49	***0.84***	**P**
P1	0.52	-	-	0.48	0.57	-	-	-	-	-	***0.73***	***0.62***	-	-	0.45	***0.59***	0.48	-	***0.66***	0.29	0.44	-	***0.82***		-	-	-	-	-	**P1**
P2	0.34	-	-	0.57	***0.76***	-	-	-	-	-	0.41	0.45	-	-	0.04	0.51	0.57	-	0.57	***0.76***	***0.81***	-	***0.59***	0.55		0.48	0.48	0.56	***0.91***	**P2**
P3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		***1.00***	-0.22	0.48	**P3**
P4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-0.22	0.48	**P4**
PER	0.39	-	-	0.48	***0.74***	-	-	-	-	-	0.22	0.40	-	-	0.22	0.42	-0.09	-	0.31	0.39	0.40	-	0.39	0.31	0.47	-	-		0.46	**PER**
PY	***0.70***	-	-	0.50	0.04	-	-	-	-	-	***0.61***	0.26	-	-	***0.69***	0.49	-0.31	-	0.54	0.09	0.04	-	0.55	0.49	0.13	-	-	0.31		**PY**
	AN	**Ace**	**BaA**	**C**	**C1**	**C2**	**D**	**D1**	**D2**	**D3**	**F**	**F1**	**F2**	**F3**	**FL**	**FP1**	**FP2**	**N**	**N1**	**N2**	**N3**	**N4**	**P**	**P1**	**P2**	**P3**	**P4**	**PER**	**PY**	

2013

Table 5. Results from Dunn non-parametric multiple comparisons of PAH concentrations among sites for juvenile mussels by year, 2012 and 2013. Within a column, sites with different letters indicate significant differences (i.e., where Bonferonni-adjusted p-values ≤ 0.05). Only those PAHs with significant pairwise differences are included here.

Site	Ace	BaA	C1	C2	D1	D3	F1	FL	FP1	FP2	N	N3	N4	P	P3	P4	PER	PY	Site
IC	ab	ab	ac	a	a	a	ab	ab	ab	a	ab	ab	ab	ab	a	a	a	ab	IC
ART	ab	ab	b	a	a	a	a	ab	ab	a	ab	ab	ab	ab	a	a	ab	ab	ART
CLE	ab	ab	b	a	a	a	ab	ab	ab	a	ab	ab	ab	ab	a	a	ab	ab	CLE
DC	ab	ab	bc	a	a	a	ab	ab	ab	a	ab	ab	ab	ab	a	a	ab	ab	DC
CAR	a	ab	b	a	a	a	ab	ab	ab	a	ab	ab	ab	ab	a	a	ab	ab	CAR
GR	ab	a	a	b	b	b	b	ab	ab	b	a	ab	a	a	b	b	ab	ab	GR
SIM	b	a	b	a	a	a	b	a	a	a	ab	a	a	ab	a	a	ab	a	SIM
PEN	b	a	b	a	a	a	b	ab	ab	a	ab	ab	a	ab	a	a	ab	ab	PEN
CHP	ab	ab	b	a	a	a	ab	ab	ab	a	a	ab	ab	a	a	a	ab	ab	CHP
CC	ab	ab	bc	a	a	a	ab	b	ab	a	ab	b	ab	ab	a	a	ab	ab	CC
HF	ab	ab	b	a	a	a	a	ab	ab	a	ab	ab	ab	ab	a	a	a	ab	HF
WB	ab	a	b	a	a	a	ab	ab	b	a	b	ab	b	b	a	a	b	b	WB

Table 6. Spearman's rho correlation coefficients for correlations between site-specific June and October 2013 pore water metal concentrations and 2013 juvenile mussel metal tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from analyses.

Metal	*June 2013*	*October 2013*
Al	-0.30	0.20
As	BDL	0.38
Ba	0.11	0.03
Be	BDL	BDL
Cd	BDL	BDL
Co	0.45	0.15
Cr	*BDL*	0.33
Cu	0.01	-0.42
Fe	0.15	0.18
Hg	BDL	BDL
K	BDL	0.34
Mg	***0.68****	***0.66****
Mn	0.05	-0.08
Mo	0.24	-0.04
Ni	-0.27	-0.09
Pb	-0.43	0.14
Sb	BDL	BDL
Se	BDL	BDL
Si	BDL	BDL
Sr	0.01	-0.01
V	-0.43	0.29
Zn	-0.36	***0.50***

Table 7. Spearman's rho correlation coefficients for correlations between site-specific bed (2012) and particulate (2013) sediment and juvenile mussel metal tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from our analyses.

Metal	2012 Juveniles *Bed*	2013 Juveniles *Bed*	2013 Juveniles *Particulate*
Al	-0.09	***0.53***	0.12
As	0.34	0.02	***0.74****
Ba	-0.22	-0.12	0.28
Be	BDL	BDL	BDL
Cd	BDL	BDL	BDL
Co	-0.27	0.27	0.43
Cr	-0.22	***0.54***	0.05
Cu	0.01	0.14	-0.01
Fe	-0.29	-0.01	0.19
Hg	BDL	BDL	BDL
K	-0.27	0.27	0.30
Mg	-0.14	-0.12	-0.22
Mn	0.22	-0.11	0.34
Mo	BDL	BDL	BDL
Ni	***-0.52***	0.20	***-0.58***
Pb	-0.04	0.38	0.10
Sb	BDL	BDL	BDL
Se	BDL	BDL	BDL
Si	-0.01	0.47	0.27
Sr	-0.05	-0.31	0.14
V	0.15	0.16	0.11
Zn	0.14	-0.09	-0.22

Table 8. Spearman's rho correlation coefficients for correlations between site-specific surface water concentrations of metals (by sampling event) and juvenile mussel metal tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from our analyses. Sampling events were as follows: Q1 = August 21-23, 2012 (mainstem only); Q2 = October 24-25, 2012 (tributaries) and November 15-16, 2012 (mainstem); Q4 = July 18-19, 2013 (mainstem) and August 5-6, 2013 (tributaries); Q5 = September 18-20, 2013 (mainstem only). Juvenile mussels deployed from June 5-6, 2012 to October 24-25, 2012 and July 31-August 1, 2013 to November 5-7, 2013.

	2012		*2013*	
Metal	Q1	Q2	Q4	Q5
Al	0.45	-0.24	0.42	***0.72****
Cr	BDL	BDL	-0.41	-0.35
Cu	BDL	BDL	-0.22	BDL
Fe	0.43	BDL	0.06	***0.78****
K	0.19	-0.17	0.47	***0.79****
Mg	***-0.77****	0.16	***0.58***	***0.73****
Mn	0	***0.68****	0.14	***0.86****
Ni	-0.35	-0.24	-0.09	0.09
Zn	0.32	BDL	0.26	0.14

Table 9. Spearman's rho correlation coefficients for correlations between site-specific June and October 2013 pore water PAH concentrations and 2013 juvenile mussel PAH tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from analyses.

PAH	*June 2013*	*October 2013*
AN	0.06	BDL
C	-0.15	BDL
F	0.26	BDL
FL	0.49	BDL
FP1	0.09	BDL
N1	0.27	0.38
N2	0.09	BDL
N3	0.48	BDL
P	BDL	0.45
P1	***0.75****	0.21
PY	0.38	BDL
Sum34	0.27	***0.69****
Sum42	0.27	***0.69****

Table 10. Spearman's rho correlation coefficients for correlations between site-specific bed (2012) and particulate (2013) sediment and juvenile mussel PAH tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from our analyses.

PAH	2012 Juveniles *Bed*	2013 Juveniles *Bed*	2013 Juveniles *Particulate*
AN	0.35	0.41	0.41
Ace	0.10	BDL	BDL
BaA	0.39	BDL	BDL
C	0.24	0.42	0.21
D	0.04	BDL	BDL
F	0.19	***0.67****	***0.83****
F1	***0.57***	BDL	BDL
F2	0.42	BDL	BDL
F3	0.35	BDL	BDL
FL	0.38	0.45	0.31
FP1	0.22	***0.53***	***0.63****
N	0.25	BDL	***0.75****
N1	0.13	***0.69****	BDL
N2	0.39	0.48	0.49
N3	***0.65****	0.62	***0.56***
N4	***0.51***	BDL	BDL
P	0.11	***0.80****	***0.88****
P1	BDL	***0.74****	***0.81****
P2	0.21	BDL	BDL
PER	-0.17	BDL	BDL
PY	0.21	0.45	***0.62****
Sum34	0.23	***0.72****	***0.79****
Sum42	0.23	***0.73****	***0.79****

Table 11. Spearman's rho correlation coefficients for correlations between site-specific surface water concentrations of PAHs (by PSD deployment period) and juvenile mussel PAH tissue concentrations. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the PAH was excluded from our analyses. Seasonal deployments of PSDs were as follows: 2012 summer = July 18 – August 21; 2012 fall = September 27 – October 24; 2013 spring = May 7 – June 5; 2013 summer = July 30 – August 26; 2013 fall = October 8 – November 5. Juvenile mussels deployed from June 5/6, 2012 to October 24/25, 2012 and from July 31/August 1, 2013 to November 5 – 7, 2013.

PAH	2012 Summer	2012 Fall	2013 Spring	2013 Summer	2013 Fall
Ace	***0.51***	***0.97****	BDL	BDL	BDL
AN	BDL	***0.73****	***0.58***	***0.94****	0.45
BaA	***0.64****	***0.99****	BDL	BDL	BDL
C	BDL	***0.96****	0.05	0.20	0.48
D	BDL	***0.80****	BDL	BDL	BDL
F	BDL	***0.95****	0.10	***0.71****	0.25
F1	BDL	***0.66****	BDL	BDL	BDL
F2	BDL	***0.90****	BDL	BDL	BDL
F3	BDL	BDL	BDL	BDL	BDL
FL	***0.74****	BDL	-0.02	***0.70****	0.29
FP1	BDL	***1.00****	0.29	0.35	0.22
N	***0.97****	BDL	BDL	BDL	BDL
N1	0.38	BDL	0.05	0.30	0.48
N2	BDL	BDL	0.18	0.42	***0.64****
N3	BDL	***0.92****	***0.73****	***0.71****	***0.74****
N4	***0.54***	***0.71****	BDL	BDL	BDL
P	BDL	***0.89****	0.35	0.43	0.17
P1	BDL	BDL	0.51	***0.55***	0.34
P2	BDL	***0.81****	BDL	BDL	BDL
PER	BDL	0.22	BDL	BDL	BDL
PY	BDL	***0.96****	-0.11	0.28	0.39
Sum34	***0.69****	***0.98****	0.48	0.45	***0.55***
Sum42	***0.64****	***0.98****	0.48	0.45	***0.57***

Table 12. Spearman's rho correlation coefficients from correlations of metal concentrations in juvenile mussel tissue between years (2012 and 2013). Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from our analyses.

Metal	2012 x 2013
Al	-0.18
As	***0.87****
Ba	***0.81****
Be	BDL
Cd	BDL
Co	0.25
Cr	0.44
Cu	0.28
Fe	***0.78****
Hg	***0.83****
K	0.49
Mg	***0.82****
Mn	***0.90****
Mo	BDL
Ni	0.52
Pb	0.18
Sb	BDL
Se	***0.76****
Si	-0.18
Sr	***0.94****
V	-0.31
Zn	***0.85****

Table 13. Spearman's rho correlation coefficients from correlations of PAH concentrations in juvenile vs. resident mussel tissue in 2012 and 2013. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from our analyses.

PAH	2012	2013
Ace	***0.95****	BDL
AN	***0.85****	***0.93****
BaA	***0.91****	BDL
C	***0.92****	***0.95****
D	0.45	BDL
F	***0.77****	***0.81****
F1	***1.0****	BDL
F2	***0.98****	BDL
F3	***0.98****	BDL
FL	***0.87****	***0.83****
FP1	***0.84****	***1.0****
N	***0.78****	BDL
N1	-0.08	***0.90****
N2	***0.99****	***0.93****
N3	***0.93****	***0.91****
N4	***0.95****	BDL
P	***0.95****	***0.76****
P1	BDL	***0.84****
P2	***0.90****	BDL
PER	***0.72****	BDL
PY	***0.88****	***0.89****
Sum 32	***0.85****	***0.96****
Sum 42	***0.85****	***0.96****

Table 14. Spearman's rho correlation coefficients from correlations of PAH concentrations in juvenile mussel tissue between years (2012 and 2013). Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$).

PAH	2012 x 2013
AN	***0.87****
C	***0.71****
F	0.36
FL	***0.82****
FP1	0.21
N1	-0.22
N2	0.47
N3	***0.70****
P	0.33
PY	***0.55***
Sum34	***0.50***
Sum42	***0.50***

Table 15. Spearman's rho correlation coefficients from correlations of metal concentrations in juvenile vs. resident mussel tissue in 2012 and 2013. Bold and Italics with * = significant ($p \leq 0.05$); Bold and Italics only = marginal significance ($0.05 < p < 0.10$). BDL = more than half of samples were below detection limit; therefore, the metal was excluded from our analyses.

Metal	2012	2013
Al	***0.92****	0.42
As	-0.35	-0.01
Ba	-0.23	-0.24
Be	BDL	BDL
Cd	BDL	0.48
Co	0.47	0.32
Cr	***0.64****	-0.08
Cu	0.25	-0.26
Fe	0.30	-0.52
Hg	***-0.61***	0.20
K	***0.72****	-0.36
Mg	0.15	0.04
Mn	0.28	0.16
Mo	***0.70****	BDL
Ni	0.13	0.24
Pb	***0.89****	-0.14
Sb	BDL	-0.32
Se	0.49	0.24
Si	***0.92****	0.39
Sr	0.38	-0.02
V	***0.84****	0.15
Zn	0.48	-0.01

Table 16. Results from correlation analyses of site-specific mean total mussel density vs. metal concentrations measured in juvenile mussel tissues in 2012 and 2013. r_s is the Spearman rho correlation coefficient. Mussel densities were based on quantitative quadrat surveys conducted intermittently over the years 2005-2014 by Krstolic et al. 2013, Jones et al. 2014, and J. Jones (personal communication).

	2012 Juveniles		*2013 Juveniles*	
Metal	r_s	p-value	r_s	p-value
Al	-0.64	0.09	0.50	0.21
As	0.07	0.87	-0.69	0.06
Ba	-0.31	0.46	-0.38	0.35
Cd	0.17	0.69	-0.35	0.40
Co	-0.62	0.10	0.14	0.74
Cr	-0.50	0.21	0.43	0.29
Cu	-0.52	0.18	0.57	0.14
Fe	-0.60	0.12	0.21	0.61
Hg	0.12	0.78	0.14	0.74
K	-0.79	0.02	0.12	0.78
Mg	-0.24	0.57	0.00	1.00
Mn	0.26	0.53	0.19	0.65
Mo	-0.60	0.12	-0.05	0.91
Ni	-0.69	0.06	0.31	0.46
Pb	-0.62	0.10	0.45	0.26
Sb	-0.86	0.01	-0.19	0.65
Se	-0.33	0.42	-0.45	0.26
Si	-0.62	0.10	0.62	0.10
Sr	-0.55	0.16	-0.24	0.57
V	-0.52	0.18	0.10	0.82
Zn	0.24	0.57	-0.40	0.32

Table 17. Results from correlation analyses of site-specific mean total mussel density vs. PAH concentrations measured in deployed juvenile mussel tissues in 2012 and 2013. r_s is the Spearman rho correlation coefficient, and "BDL" indicates that PAH concentrations were below detection limits at more than half the sites and therefore, the PAH was not included in analyses. Mussel densities were based on quantitative quadrat surveys conducted intermittently over the years 2005-2014 by Krstolic et al. 2013, Jones et al. 2014, and J. Jones (personal communication).

	2012 Juveniles		*2013 Juveniles*	
PAH	r_s	p-value	r_s	p-value
Ace	-0.17	0.69	BDL	BDL
AN	-0.55	0.16	-0.57	0.14
BaA	-0.33	0.42	BDL	BDL
C	-0.33	0.42	-0.57	0.14
D	-0.16	0.70	BDL	BDL
F	-0.45	0.26	-0.67	0.07
F1	-0.93	<0.01	BDL	BDL
F2	-0.73	0.04	BDL	BDL
FL	-0.50	0.21	-0.69	0.06
FP1	-0.43	0.28	BDL	BDL
N	-0.50	0.21	BDL	BDL
N1	0.24	0.56	-0.71	0.05
N2	-0.75	0.03	-0.55	0.16
N3	-0.57	0.14	-0.55	0.16
N4	-0.57	0.14	BDL	BDL
P	-0.62	0.10	-0.62	0.10
P1	BDL	BDL	-0.71	0.05
P2	-0.33	0.42	BDL	BDL
PER	-0.40	0.32	BDL	BDL
PY	-0.40	0.32	-0.76	0.03
Sum42	-0.48	0.23	-0.90	<0.01

Figure legends

Figure 1. Study area, the Clinch River of Virginia and Tennessee, and sites where juvenile mussels were deployed. The solid black dots denote the 8 mainstem sampling sites (site names bolded), and white triangles indicate the 4 tributary sites (site names italicized). The 'Zone of Decline', downstream of Cleveland to Clinchport, represents a 68 km stretch of river where mussel populations have declined over the past 30 years (Jones et al. 2014).

Figure 2. Mussel deployment methods. (A) Cages were constructed from polyethylene industrial containers with mesh sides and base with plastic mesh (6.34 mm) affixed to the inside of the cages on the top, sides, and bottom to contain mussels and allow water to flow through the cages. Cages were deployed in areas with fine and coarse gravel and river substrate was used to fill the cages as they were partially buried to ensure the tops of the cages were flush with the sediment-water interface. (B) Concrete mussel silos were constructed to house mussels in the water column; cylindrical chambers had mesh tops and bottoms to allow water to circulate through the chamber.

Figure 3. Initial (starting) length distributions of the baseline subset of mussels (not deployed) in 2012 (top panel) and those deployed in cages (middle panel) and silos (bottom panel) in 2013. Length data were pooled across sites within each deployment method (cages or silos) in 2013.

Figure 4. Adjusted survival (percent of mussels recovered alive out of the mussels recovered) for (A) Villosa iris (2012) and (B) Lampsilis fasciola (2013). Tributary sites are denoted by asterisks. Green bar indicates sites within the 'Zone of Decline' (see Figure 1). For sites where survival in each sample was 100%, the standard error (SE) was 0 (no SE bar shown).

Figure 5. Growth rate for (A) Villosa iris (2012) and (B) Lampsilis fasciola (2013). Tributary sites are denoted by asterisks. Green bar indicates sites in the 'Zone of Decline' (see Figure 1).

Figure 6. Representative spatial trends of juvenile mussel metal concentrations. Mussels were deployed from June 5-6, 2012 to October 24-25, 2012 (black triangles) and July 31-August 1, 2013 to November 5-7, 2013 (gray triangles). Means and standard errors were based upon 4 composite samples from each site. Tributary sites are denoted by asterisks. Lines connecting points are for visual presentation and do not reflect connectivity between means.

Figure 7. Two-way dendrogram from cluster analysis of juvenile mussel (Villosa iris) metal concentrations among metals in 2012. Relative metal concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the particulate sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 21 metals that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 8. Two-way dendrogram from cluster analysis of juvenile mussel (Lampsilis fasciola) metal concentrations among metals in 2013. Relative metal concentrations are indexed by the

color gradient as darker red cells indicate higher concentrations of a given metal in the particulate sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 21 metals that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 9. Spatial trends of juvenile mussel total PAH concentrations. Mussels were deployed from June 5-6, 2012 to October 24-25, 2012 (black triangles) and July 31-August 1, 2013, to November 5-7, 2013 (gray triangles). Means and standard errors were based upon 4 composite samples from each site. Tributary sites are denoted by asterisks. Lines connecting points are for visual presentation and do not reflect connectivity between means.

Figure 10. Two-way dendrogram from cluster analysis of juvenile mussel (Villosa iris) PAH concentrations among sites in 2012. Relative PAH concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the particulate sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 12 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 11. Two-way dendrogram from cluster analysis of juvenile mussel (Lampsilis fasciola) PAH concentrations among sites in 2013. Relative PAH concentrations are indexed by the color gradient as darker red cells indicate higher concentrations of a given metal in the particulate sediment at a particular site. The 'Information Remaining' metric is the percent of the total original variation in multivariate distances among all 12 sites that remains after each joining (node) of clusters or sites. At each successive joining, some variation among sites or clusters (i.e., information) is lost until all sites are joined into one cluster and none of the original variation (information) remains.

Figure 12. Stable isotope values (δ 15N and δ 13C) for (A) Villosa iris (2012) and (B) Lampsilis fasciola (2013). Symbols represent the site average of 2 – 3 composite samples. The tributary sites are identified; CC = Copper Creek, DC = Dumps Creek, GR = Guest River, and IC = Indian Creek.

Figure 13. Boxplots illustrating the strength of association between juvenile mussel tissue concentrations of contaminants (metals and PAHs) and their corresponding concentrations in various environmental compartments (pore water, sediment, and surface water). The boxplots show the distribution of Spearman rho correlation coefficients (r_s) for each set of analyses [i.e., all correlations run for each combination of contaminant type (metals or PAHs) by environmental compartment]. For example, the second box plot shows the distribution of r_s values for all 15 correlations run between tissue and pore water concentrations of PAHs across sampling events (June and October 2013). Note for sediment that r_s values are only shown for bed sediment (not particulate).

Figure 14. Correlation between juvenile mussel total PAH tissue concentrations in Villosa iris (2012) and surface water total PAH concentrations measured via passive sampling devices (PSD) deployed in fall 2012 at all sites in the Clinch River.

Figure 15. Representative relationships between growth rate and tissue concentrations of metals in juvenile mussels (*Villosa iris*) in cages in 2012. Regression statistics are from curvilinear regressions performed in SAS.

Figure 16. Relationships between growth rate and tissue concentrations of manganese in juvenile mussels (*Villosa iris*) in cages in 2012.

Figure 17. Scatterplot of site-specific mean total mussel density (all species combined) versus total PAH concentrations measured in juvenile mussel tissues in 2012 and 2013. Mussel density data were provided by Krstolic et al. (2013), Jones et al. (2014), and J. Jones (personal communication). Sites are labeled with three letter abbreviations as follows: ART = Artrip, CAR = Carterton, CC = Copper Creek, CLE = Cleveland, CHP = Clinchport, DC = Dumps Creek, GR = Guest River, HF = Horton Ford, IC = Indian Creek, PEN = Pendleton, SIM = Simones, WB = Wallen Bend.

Figure 18. Stacked bars represent the percent contribution of each metal cluster (from two-way cluster analyses, see Figure 7) to the total metal contribution based upon standardized tissue concentrations of metals in juvenile mussels (*Villosa iris*) in cages at the mainstem sites in 2012. Blue dots represent the growth rate for *V. iris* at each site. Lines connecting points are for visual presentation and do not reflect connectivity between means.

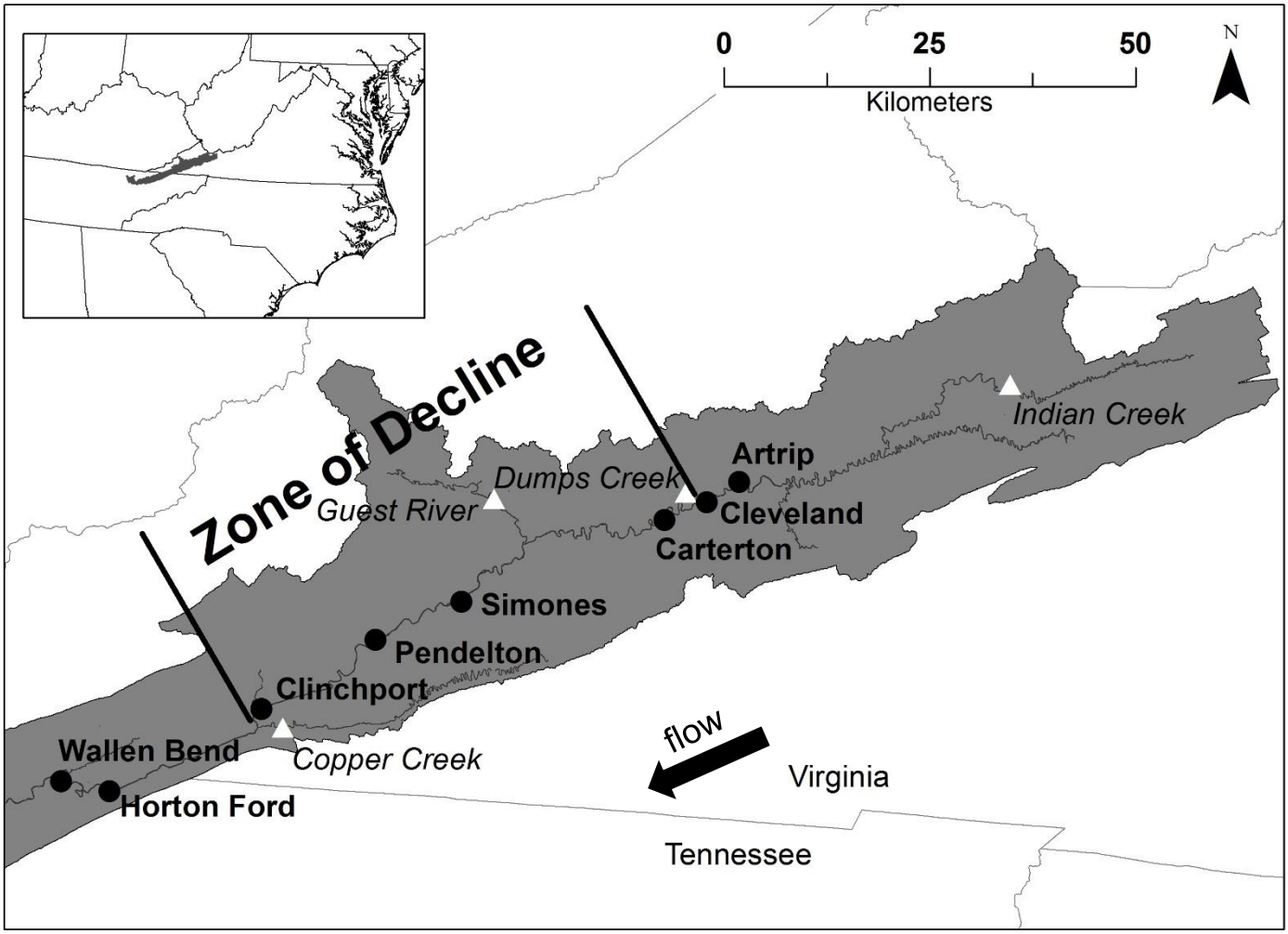
0
25
50
Kilometers
N
Zone of Decline
Guest River
Dumps Creek
Indian Creek
Artrip
Cleveland
Carterton
Simones
Pendelton
Clinchport
Copper Creek
Wallen Bend
Horton Ford
flow
Virginia
Tennessee

Figure 1.

Figure 2.

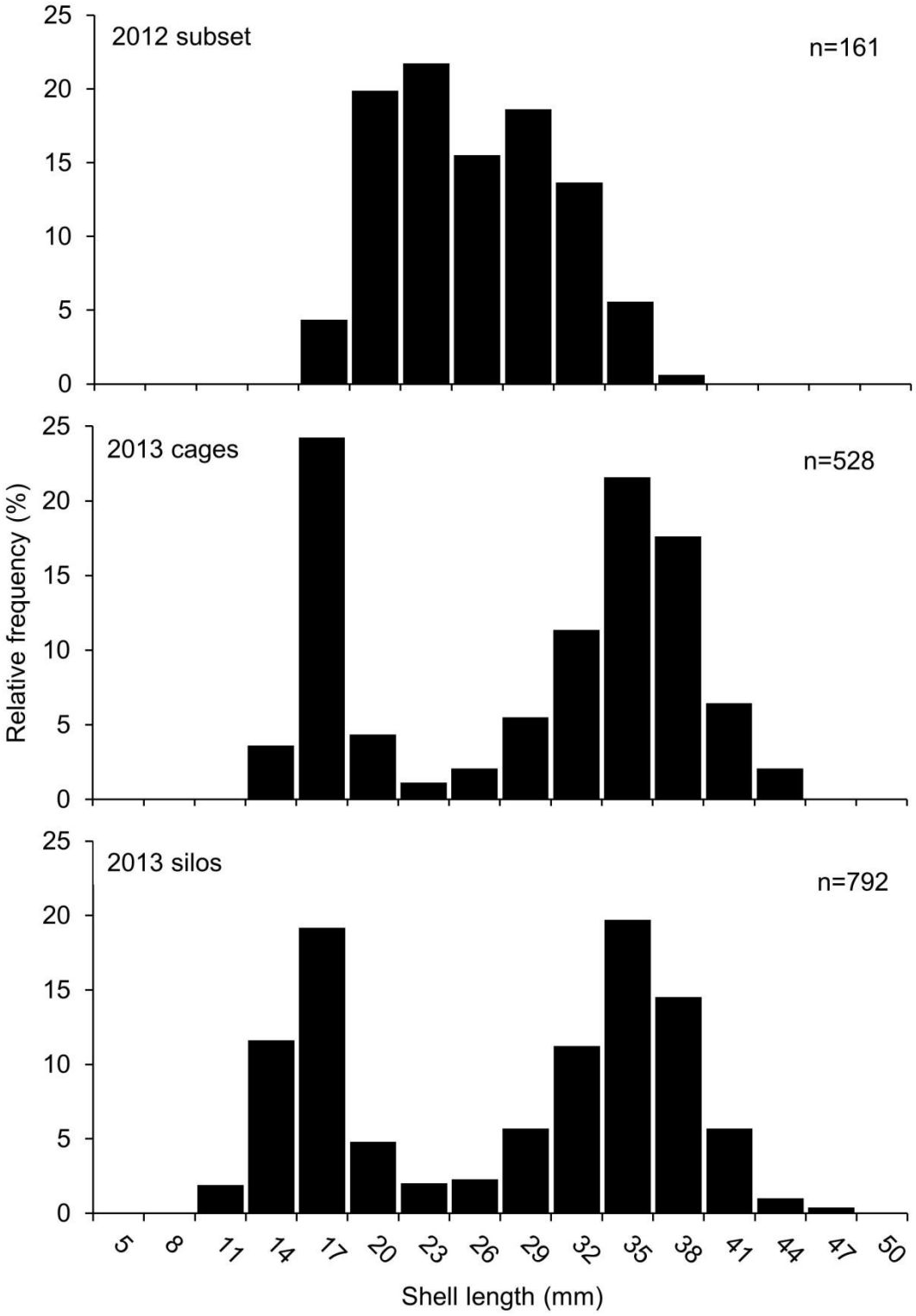
2012 subset
n=161
2013 cages
n=528
2013 silos
n=792
Relative frequency (%)
Shell length (mm)
0
5
10
15
20
25
5
8
11
14
17
20
23
26
29
32
35
38
41
44
47
50

Figure 3.

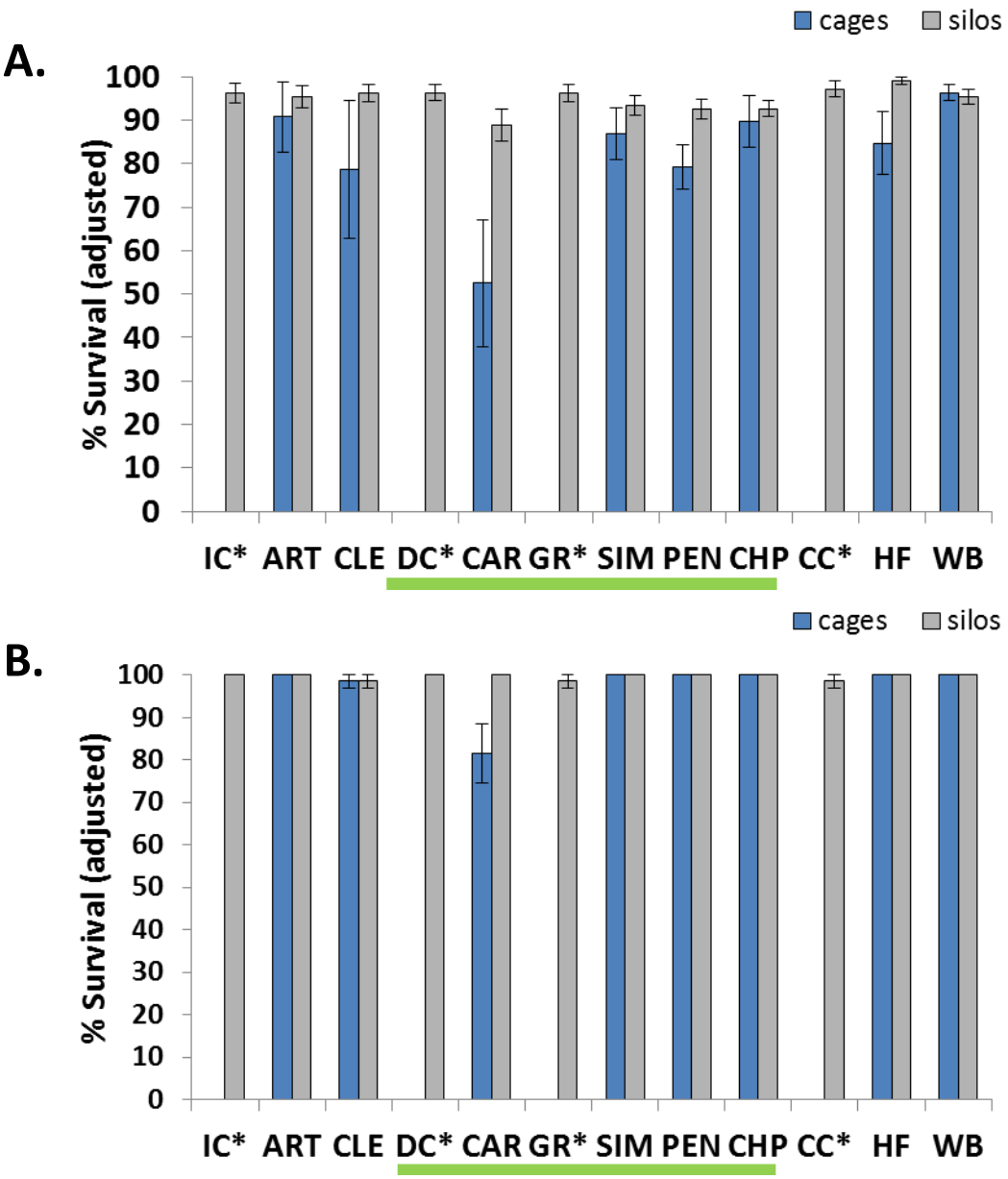
A.
cages
silos
% Survival (adjusted)
100
90
80
70
60
50
40
30
20
10
0
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB
B.
cages
silos
% Survival (adjusted)
100
90
80
70
60
50
40
30
20
10
0
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB

Figure 4.

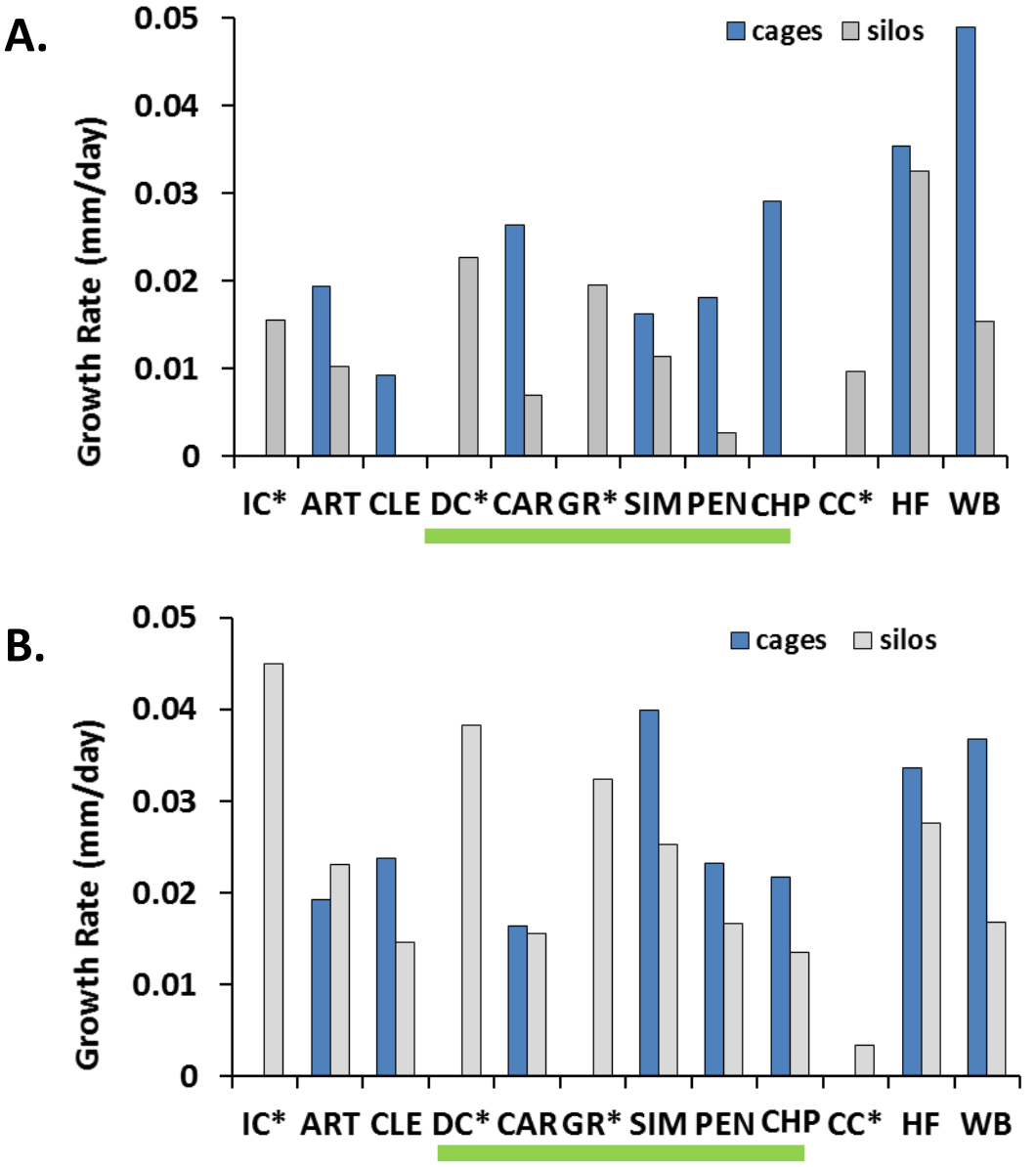
A.
0.05
0.04
0.03
0.02
0.01
0
Growth Rate (mm/day)
cages
silos
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB
B.
0.05
0.04
0.03
0.02
0.01
0
Growth Rate (mm/day)
cages
silos
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB

Figure 5.

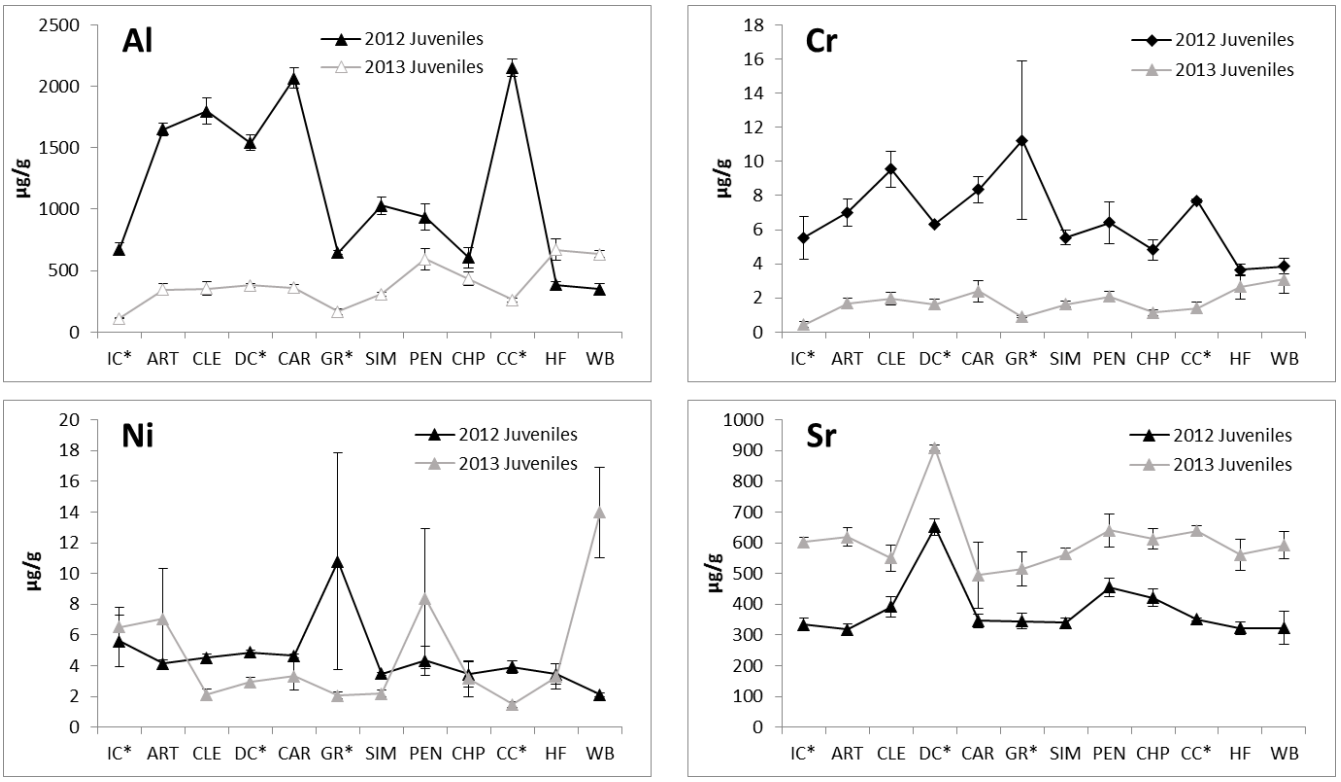
Al
2012 Juveniles
2013 Juveniles
µg/g
2500
2000
1500
1000
500
0
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB
Cr
2012 Juveniles
2013 Juveniles
µg/g
18
16
14
12
10
8
6
4
2
0
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB
Ni
2012 Juveniles
2013 Juveniles
µg/g
20
18
16
14
12
10
8
6
4
2
0
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB
Sr
2012 Juveniles
2013 Juveniles
µg/g
1000
900
800
700
600
500
400
300
200
100
0
IC* ART CLE DC* CAR GR* SIM PEN CHP CC* HF WB

Figure 6.

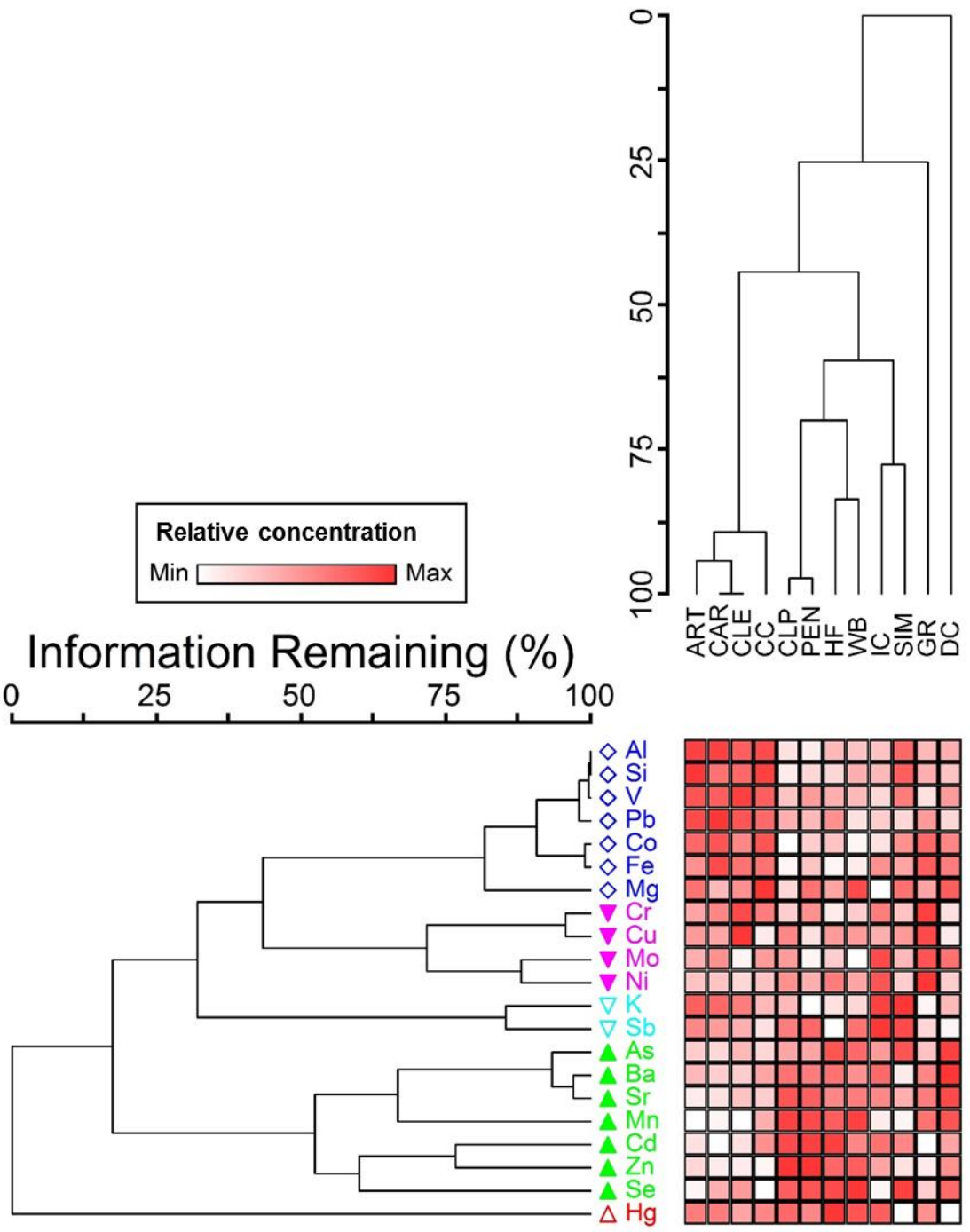
Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
ART
CAR
CLE
CC
CLP
PEN
HF
WB
IC
SIM
GR
DC
Al
Si
V
Pb
Co
Fe
Mg
Cr
Cu
Mo
Ni
K
Sb
As
Ba
Sr
Mn
Cd
Zn
Se
Hg

Figure 7.

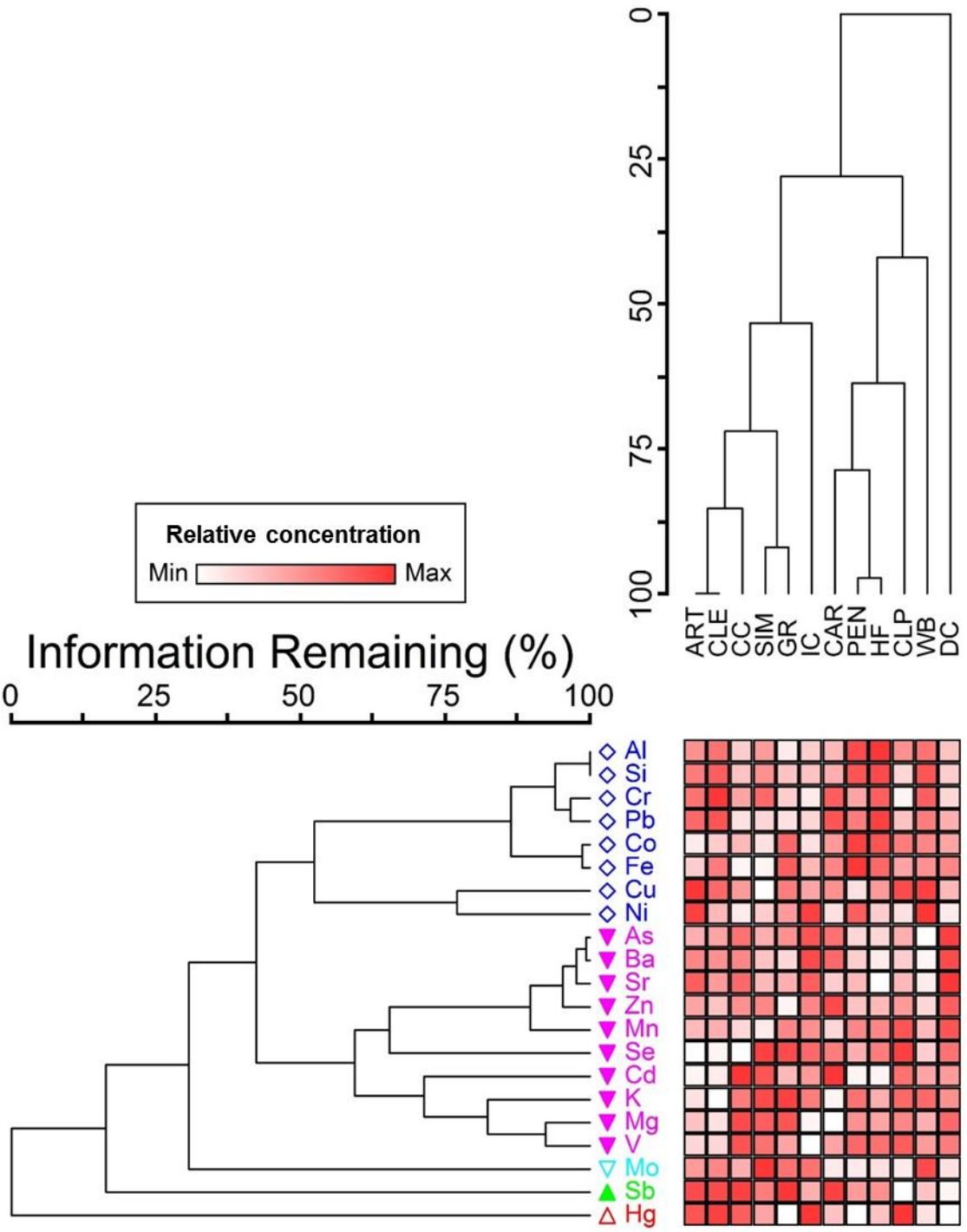
Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
ART
CLE
CC
SIM
GR
IC
CAR
PEN
HF
CLP
WB
DC
Al
Si
Cr
Pb
Co
Fe
Cu
Ni
As
Ba
Sr
Zn
Mn
Se
Cd
K
Mg
V
Mo
Sb
Hg

Figure 8.

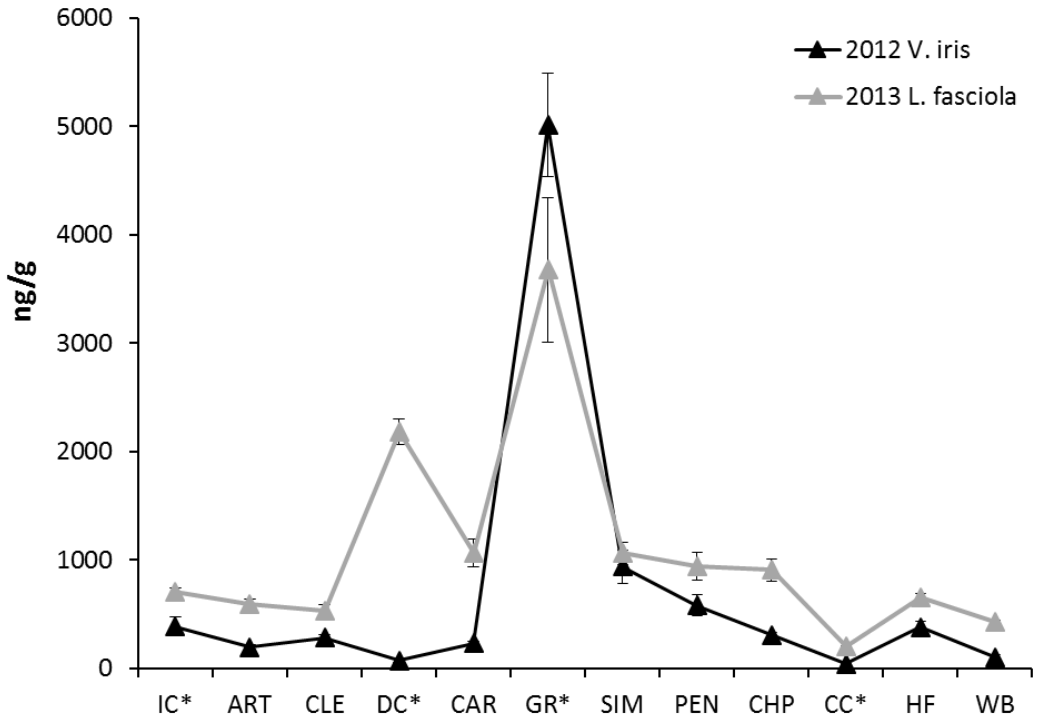
6000
5000
4000
3000
2000
1000
0
ng/g
2012 V. iris
2013 L. fasciola
IC*
ART
CLE
DC*
CAR
GR*
SIM
PEN
CHP
CC*
HF
WB

Figure 9.

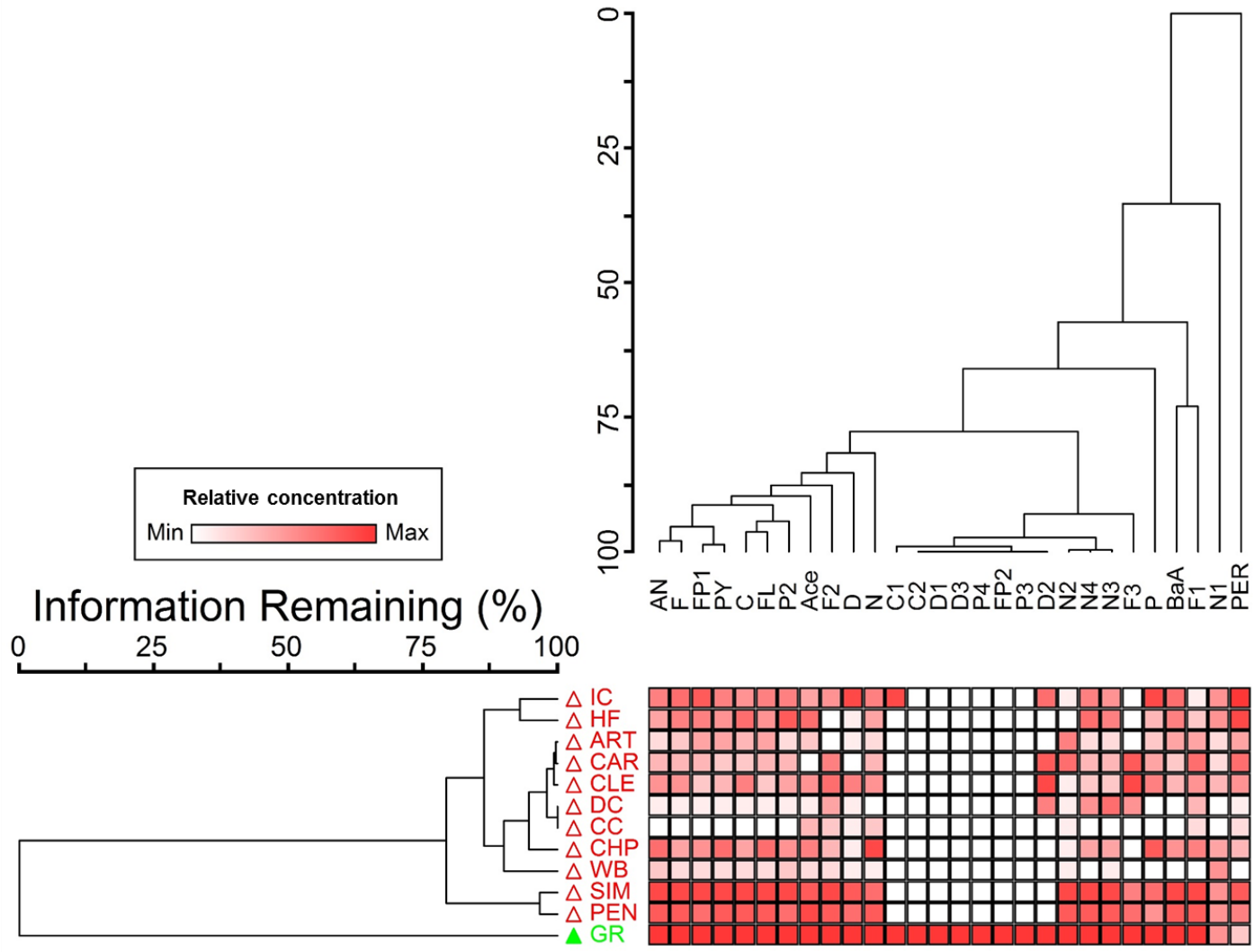
Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
AN
F
FP1
PY
C
FL
P2
Ace
F2
D
N
C1
C2
D1
D3
P4
FP2
P3
D2
N2
N4
N3
F3
P
BaA
F1
N1
PER
IC
HF
ART
CAR
CLE
DC
CC
CHP
WB
SIM
PEN
GR

Figure 10.

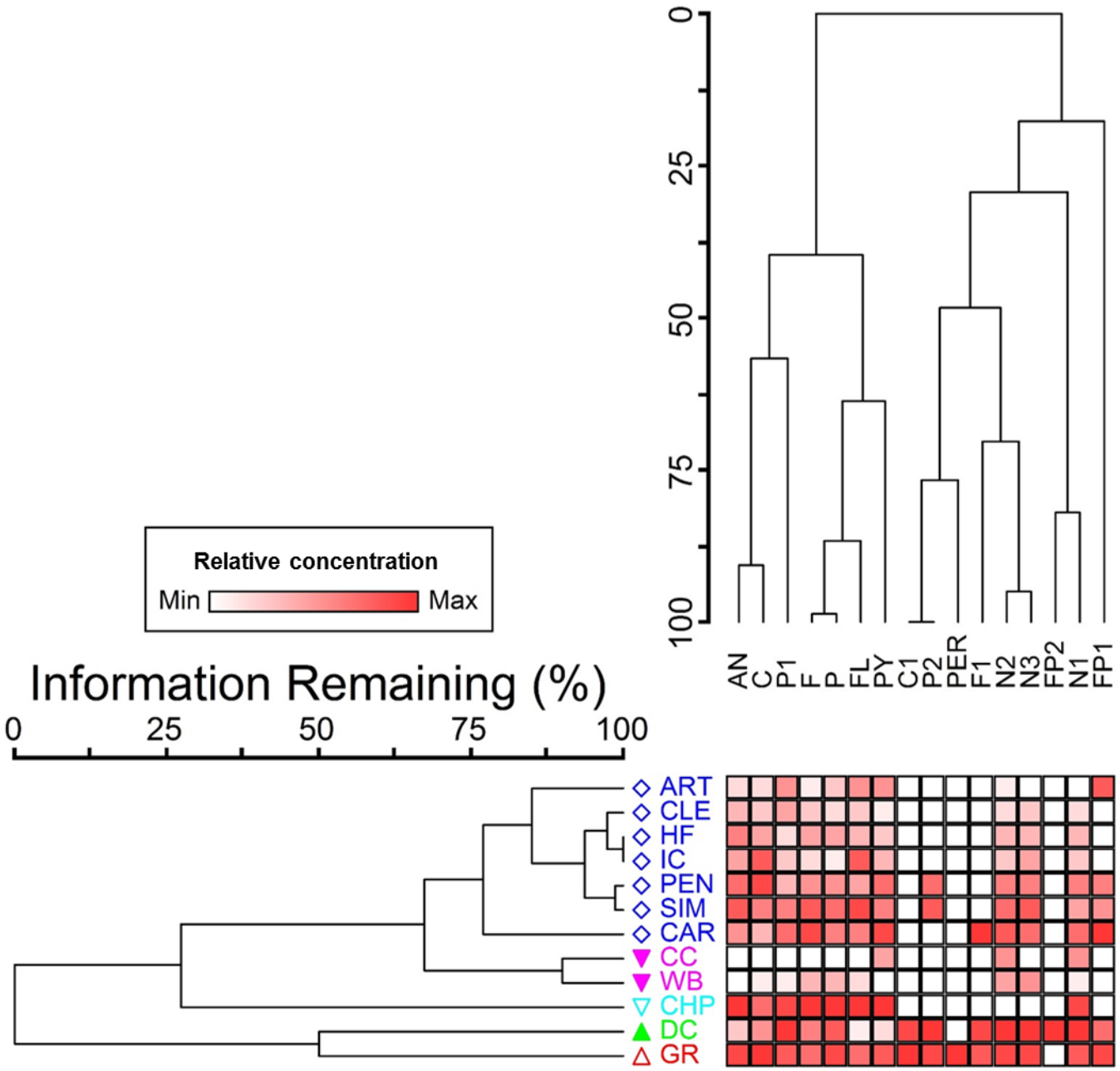
Relative concentration
Min
Max
Information Remaining (%)
0
25
50
75
100
AN
C
P1
F
P
FL
PY
C1
P2
PER
F1
N2
N3
FP2
N1
FP1
ART
CLE
HF
IC
PEN
SIM
CAR
CC
WB
CHP
DC
GR

Figure 11..

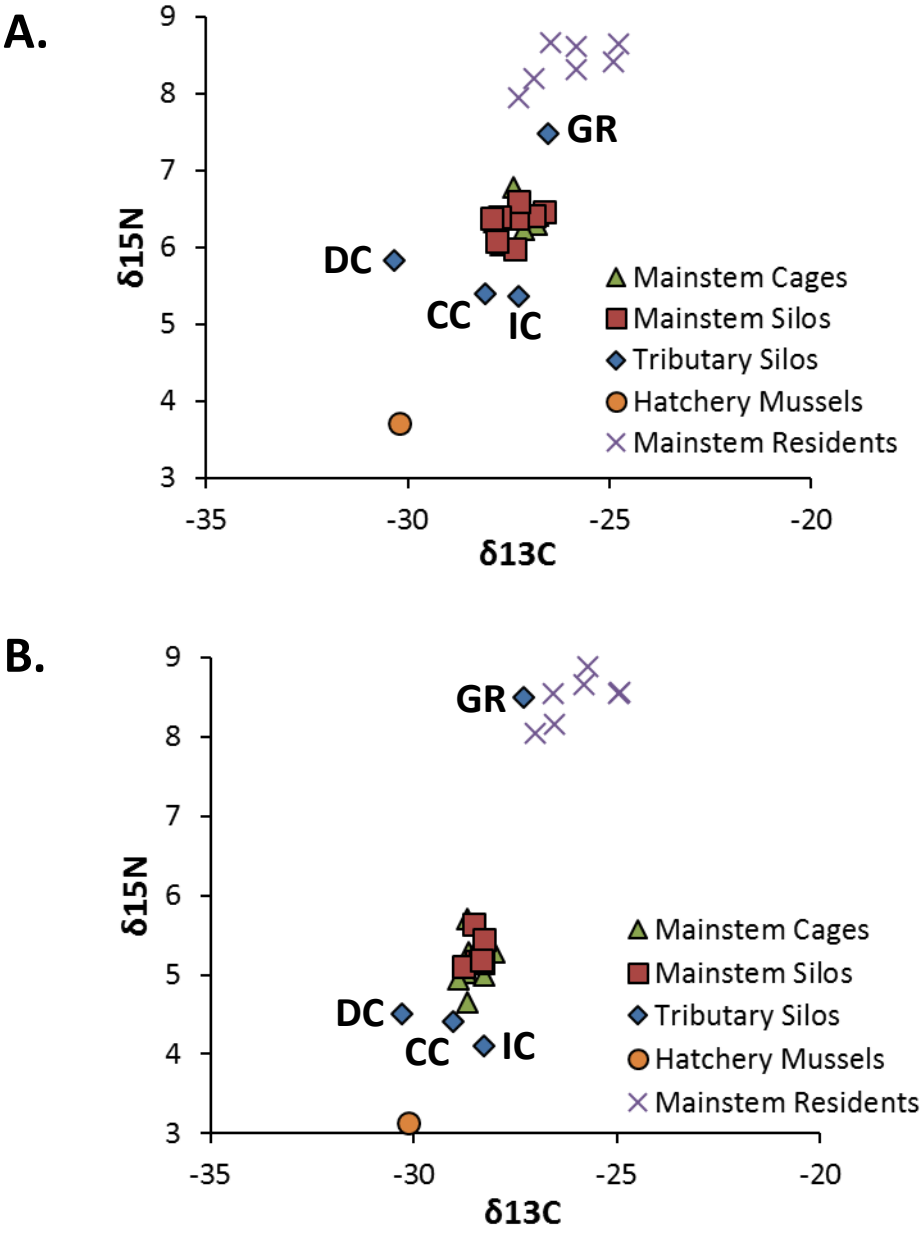
A.
9
8
7
6
5
4
3
δ15N
-35
-30
-25
-20
δ13C
GR
DC
CC
IC
Mainstem Cages
Mainstem Silos
Tributary Silos
Hatchery Mussels
Mainstem Residents
B.
9
8
7
6
5
4
3
δ15N
-35
-30
-25
-20
δ13C
GR
DC
CC
IC
Mainstem Cages
Mainstem Silos
Tributary Silos
Hatchery Mussels
Mainstem Residents

Figure 12.

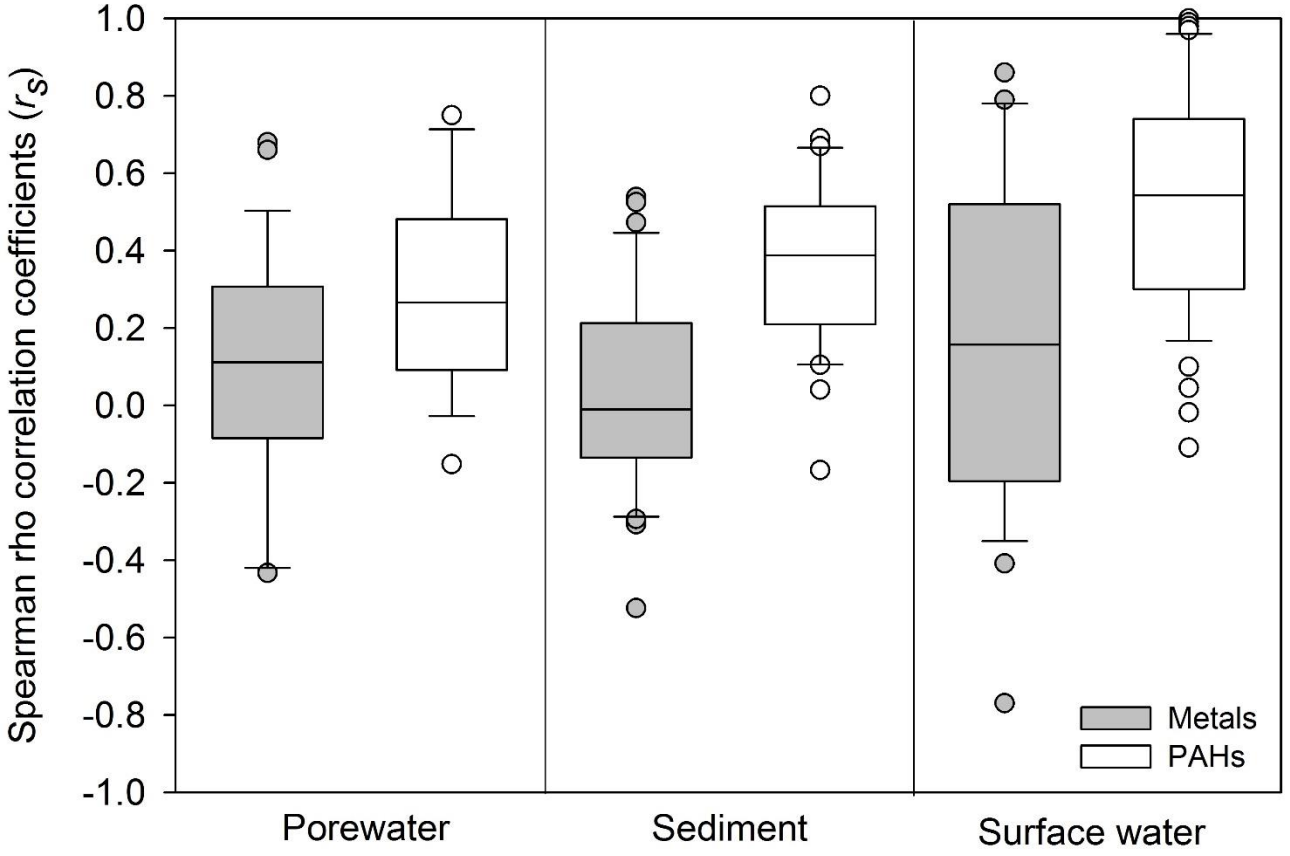
Spearman rho correlation coefficients (r_S)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Porewater
Sediment
Surface water
Metals
PAHs

Figure 13.

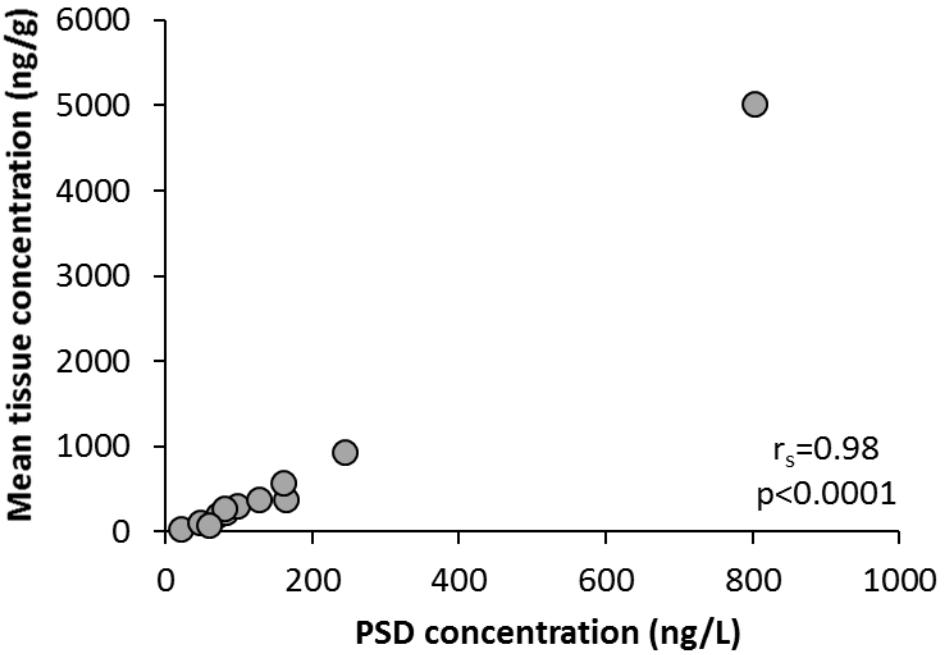
Mean tissue concentration (ng/g)
6000
5000
4000
3000
2000
1000
0
0
200
400
600
800
1000
PSD concentration (ng/L)
r_s=0.98
p<0.0001

Figure 14.

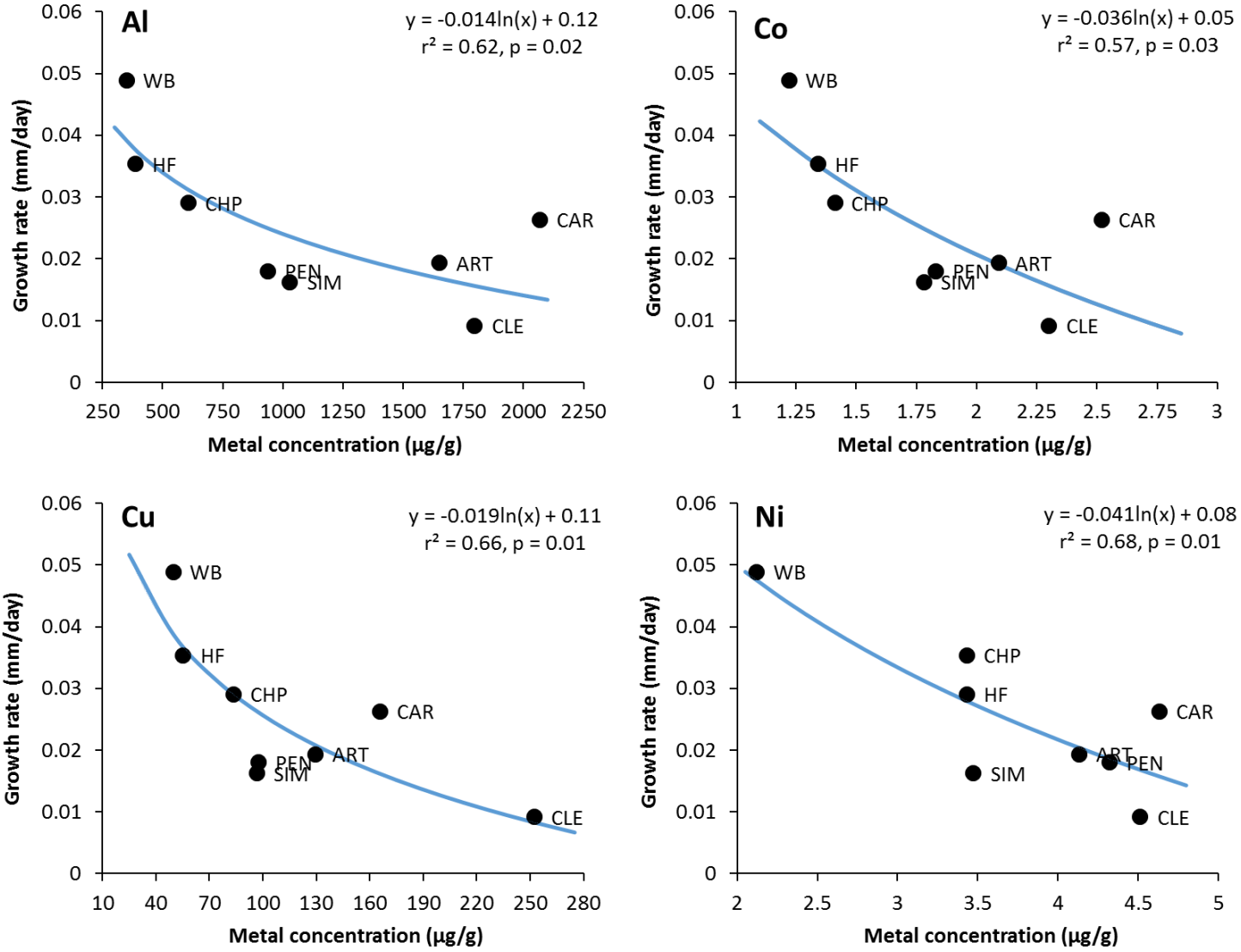
Al
y = -0.014ln(x) + 0.12
r² = 0.62, p = 0.02
Co
y = -0.036ln(x) + 0.05
r² = 0.57, p = 0.03
Cu
y = -0.019ln(x) + 0.11
r² = 0.66, p = 0.01
Ni
y = -0.041ln(x) + 0.08
r² = 0.68, p = 0.01
Growth rate (mm/day)
Metal concentration (µg/g)
WB
HF
CHP
CAR
PEN
SIM
ART
CLE

Figure 15.

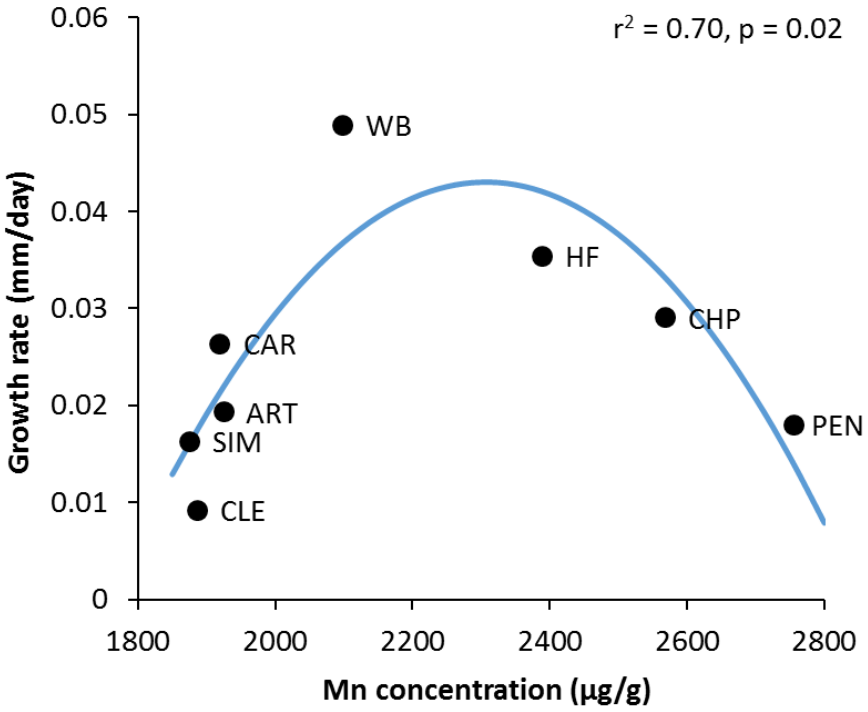
r2 = 0.70, p = 0.02
WB
HF
CHP
CAR
ART
SIM
PEN
CLE
Growth rate (mm/day)
0.06
0.05
0.04
0.03
0.02
0.01
0
1800
2000
2200
2400
2600
2800
Mn concentration (µg/g)

Figure 16.

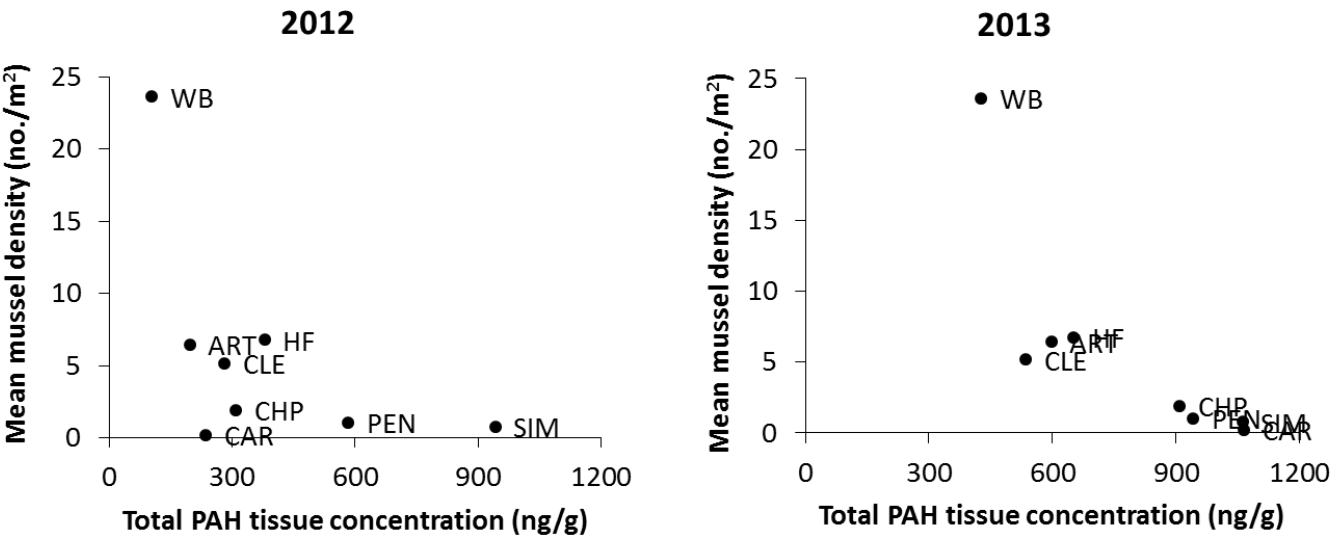
2012
Mean mussel density (no./m²)
25
20
15
10
5
0
WB
ART
HF
CLE
CHP
CAR
PEN
SIM
0
300
600
900
1200
Total PAH tissue concentration (ng/g)
2013
Mean mussel density (no./m²)
25
20
15
10
5
0
WB
CLE
CHP
0
300
600
900
1200
Total PAH tissue concentration (ng/g)

Figure 17.

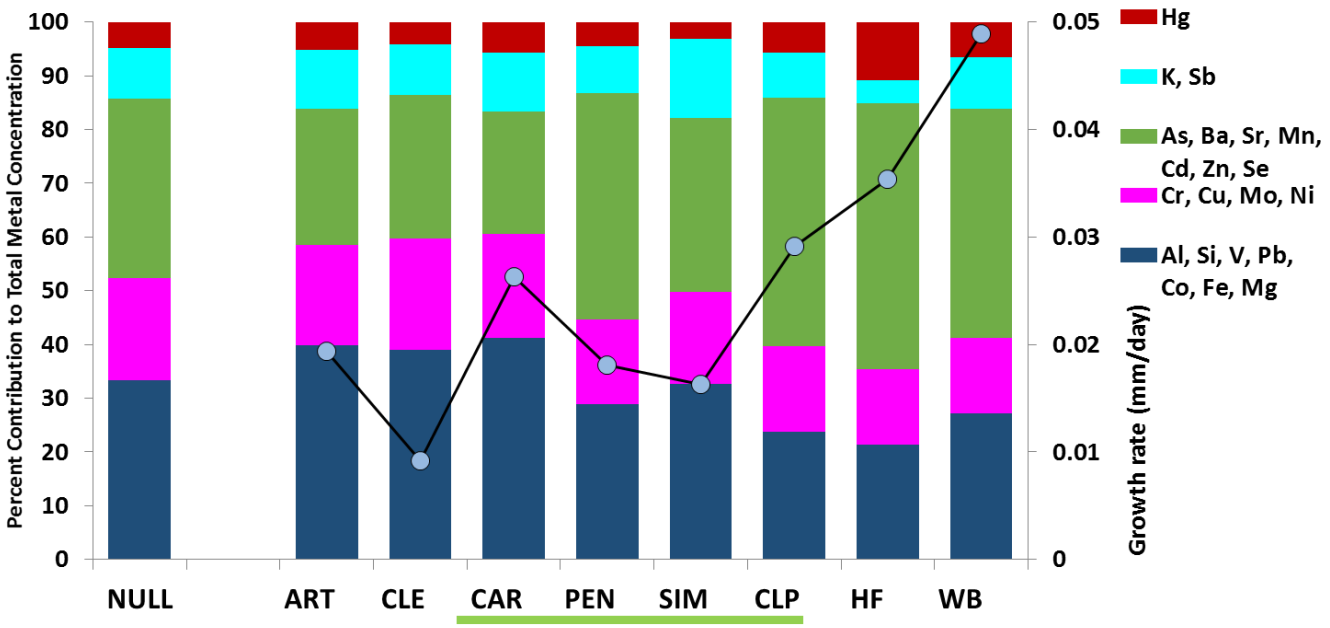
Percent Contribution to Total Metal Concentration
100
90
80
70
60
50
40
30
20
10
0
0.05
0.04
0.03
0.02
0.01
0
Growth rate (mm/day)
Hg
K, Sb
As, Ba, Sr, Mn, Cd, Zn, Se
Cr, Cu, Mo, Ni
Al, Si, V, Pb, Co, Fe, Mg
NULL
ART
CLE
CAR
PEN
SIM
CLP
HF
WB

Figure 18.

Chapter 4: Assessing toxicity of contaminants in riverine suspended sediments to freshwater mussels

Abstract

The Clinch River in Virginia and Tennessee, USA, is well known for its diverse native freshwater mussel assemblages; however, notable declines in mussel populations in recent decades have prompted much concern and subsequent research. We examined the toxicity of recently-deposited sediments on juveniles of the freshwater mussel *Epioblasma brevidens*, by collecting time-integrated sediment samples from the water column with sediment traps from 11 sites in the Clinch River Basin, including 7 sites within an 88-km reach deemed a 'mussel zone of decline'. Sediment samples were analyzed to identify presence and concentration of metals and organic contaminants. Mussels were exposed to the riverine sediments and to three control sediments for 28 d; survival, shell length, and biomass were then assessed. Sediment treatment (i.e., river location) had a significant effect on mussel survival ($p < 0.01$) and biomass ($p = 0.02$), but did not affect length ($p = 0.37$), and sediments from two of the tributaries were the most toxic. Metals and polycyclic aromatic hydrocarbons were prevalent in sediment samples collected from all sites. Manganese was significantly correlated with mussel survival and biomass, as was ammonia with survival, and total organic carbon with biomass. Landscape analysis of potential contaminant sources indicate fossil fuel mining and agriculture likely contributed to elevated manganese and ammonia, respectively. Sediments collected with sediment traps over relatively short durations of deployment can help elucidate recent contaminant influx and its potential for inducing toxicity in benthic organisms.

Keywords: Unionoida, water quality, ammonia, metals, polycyclic aromatic hydrocarbons (PAHs)

Introduction

The southeastern United States (US) is the richest region of global diversity for freshwater fish, crayfish, and mussel species (Abell et al. 2000), and is, therefore, a region of high conservation priority. However, this high regional biodiversity intersects with intense pressures of energy mining and development, urbanization and sprawl, increasingly intensive agricultural practices, and growing demands on water and other natural resources for human use. Nestled within this complex landscape, and falling within the richest faunal province (Tennessee – Cumberland) for mussels (Unionoida) and freshwater fish in North America (Haag 2010), lies the Clinch River. More than 135 freshwater species in the province have been identified as priorities for conservation, most of which are fish and mollusks (Smith et al. 2002). The Clinch River alone has supported 56 of the region's 125 mussel species and 133 of 235 known fish species (Smith et al. 2002; Zipper et al. 2014); many of those fishes are likely integral to the unique unionoid mussel life cycle, serving as hosts during the parasitic larval stage (Barnhart et al. 2008).

Currently, ~18% of the known mussel fauna are believed to be extirpated from the river – recent surveys have reported only 46 extant species (Jones et al. 2014). In total, at least 48 freshwater mussel and fish species in the Clinch River are imperiled or vulnerable, and many of those are federally-listed as endangered or threatened (Master et al. 1998), including 20 endangered mussel species (Jones et al. 2014). Much of this decline has been documented over the last 35 years (Jones et al. 2014), a relatively short period considering the long history of human settlement in the Appalachians and the decades-long life span of many mussel species. Interestingly, the patterns of decline are not consistent throughout the Clinch River – healthier populations of mussels persist both upstream and downstream of an identified zone of mussel

decline that stretches 88 km, from Dumps Creek near the town of Carbo, Virginia (USA; river km 431) to around Clinchport, Virginia (USA; river km 343), where mussel density at some sites has declined by an alarming 96% (Jones et al. 2014; Figure 1).

Investigations into possible reasons for such declines were recently published in a series of journal articles and summarized by Zipper et al. (2014). Physical habitat was ruled out as a limiting factor, and pollution was the over-arching theme. Analyses of datasets for water and sediment quality spanning the 1960s to 2013 indicated that excessive ammonia from poor wastewater treatment was a major issue in the 1970s-1980s, though surface water concentrations have improved considerably, especially in the most recent decade (Price et al. 2014). Metal concentrations, including those released in two disastrous spills from a coal-fired power plant on the banks of the Clinch River near Carbo, were highest in the 1980s-1990s and have declined over time (Price et al. 2014), but some metals may still be a cause of concern (Johnson et al. 2014). Unlike ammonia and metals, dissolved solids, major ions, and specific conductance have trended upward over time, and were negatively associated with mussel assemblage quality (Johnson et al. 2014, Price et al. 2014, Zipper et al. 2014). Although polycyclic aromatic hydrocarbons (PAHs) were not correlated with patterns of decline in recent analyses, organic contaminants, especially coal-associated PAHs, can be found in high concentrations in much of the river, and coal particles may comprise up to 6.5% of bed sediment contents (Johnson et al. 2014, Zipper et al. 2014).

Despite difficulty in empirically linking some of the specific parameters discussed above, mining, agriculture, and urban development are often associated with poorer faunal assemblages. For example, mining-influenced tributaries received significantly lower scores in an ecotoxicological rating system than did agriculture-influenced streams, largely due to poorer

benthic macroinvertebrate communities found in streams affected by mining activities (Locke et al. 2006). Negative effects of Appalachian coal mining are not limited to pollution; the construction of valley fills from mountaintop mining practices also results in alteration of hydrology and water temperature regimes (Griffith et al. 2012), which may also impact aquatic fauna. Benthic community metrics and fish index of biotic integrity scores were lowest in coal-mining watersheds in another study, though near-stream agriculture and urban areas (within 200 m) were also identified as stressors to benthic and fish communities (Diamond et al. 2002). Given the myriad of historic and ongoing pollutant issues in the Clinch River, identifying a "smoking gun" for suffering mussel populations, along with other fauna, has proven difficult, but there is consensus that chronic toxicity arising from non-point sources and potentially from toxic mixtures is the most likely explanation.

Several studies involving the Clinch River and its watershed have focused on surface water quality, bed sediments, physical habitat, and land use. While some water quality parameters are no doubt related to runoff and sedimentation, no study has specifically addressed the contribution of such attributes from the sediment load, and we are unaware of any other aquatic contaminant investigations that specifically address this ecological compartment. The objectives of this study were to (1) determine whether fine particulate sediments in the water column (i.e., the sediment load) are toxic by collecting this material in sediment traps and conducting a 28-d laboratory exposure with freshwater mussels, (2) identify contaminants present in the sediment load and quantify their concentrations, (3) determine which contaminants are related to any toxicity observed in mussels, and (4) consider our results in the context of the long history of research in the Clinch River related to faunal decline and pollutants in other ecological compartments, such as surface water and bed sediments.

Methods

Study area

Twelve sites in the Clinch River basin were selected based on patterns of recently observed declines in mussel abundance and species richness (Jones et al. 2014). Eight mainstem river and four tributary sites were distributed upstream of (n = 2 mainstem, 1 tributary), within (n = 4 mainstem, 3 tributary), and downstream (n = 2 mainstem) of the zone of mussel decline (Figure 1). Indian Creek (IN; a tributary), Artrip (ART), and Cleveland (CLE) are located upstream; Dumps Creek (DC; a tributary), Carterton (CAR), Guest River (GR; a tributary), Simones (SIM), Pendleton (PEN), Clinchport (CHP), and Copper Creek (CC; a tributary) are within the zone of decline; and Horton Ford (HF) and Wallen Bend (WB) are downstream of the zone of decline. Copper Creek enters the Clinch River from the southeast and drains the Ridge and Valley physiographic landforms, while Indian and Dumps Creeks and the Guest River enter from the northwest, draining the coal-rich Appalachian Plateau physiographic region. The Guest River currently does not support mussels (Johnson et al. 2014), though as a major tributary, it drains a substantial portion of the Clinch River watershed.

Sediment collection and processing

Sediment traps were fashioned from 30.5-cm lengths of 7.6-cm diameter PVC pipe, had a removable bottom (bottom half of a 1-L plastic beverage bottle) fastened on with a hose clamp, and were topped with a sheet of plastic 1-cm mesh, also fastened on with a hose clamp, to keep out large debris. Two sediment traps were located at each site, with each trap secured within the openings of a two compartment cement block that was held in place to the stream bottom by rebar. Traps were designed with an optimal aspect ratio for collecting sediments in lotic

conditions (Hargrave and Burns 1979) and deployed for approximately five months (late August 2013 – late January 2014), providing a time-integrated particulate sediment sample with sufficient volume for the experimental design and contaminant analyses. Sediment samples were placed in 950-mL amber glass jars certified to meet/exceed US Environmental Protection Agency (EPA) standards for metal, pesticide, and semi-volatile analysis (Thermo Scientific ™ ICHEM series, Rockwood, Tennessee, USA) and immediately transported on ice to the laboratory, where they were transferred to an incubator and held at 4°C. Sediment samples were homogenized, passed through a 2-mm sieve (no addition of water) to remove debris and large particles, sampled for particle size, total organic content characterization, and contaminant analysis (ASTM 2008). Afterward, they were returned to the incubator for a total holding time of two weeks before test initiation to ensure removal of indigenous organisms.

Experimental design and conditions

We evaluated the toxicity of Clinch River sediments to juvenile freshwater mussels in a 28-d static-renewal exposure with 200 mL of test sediments and 800 mL reconstituted hard water (ASTM 2007) in each of four replicate 1-L beakers per treatment. Sediment and water were added to the test chambers and allowed to settle for 3 d to avoid exposing mussels to turbid conditions; mussels were added to three of the replicates afterward (= test day 0). One replicate did not contain mussels in order to evaluate potential contaminant loss via uptake by mussels vs. leaching into the dissolved fraction of overlying water. Polyethylene and universal passive sampling devices (PSDs) (Hewitt et al. 2006, Hirons 2009) were randomly placed into one replicate of each treatment to measure the dissolved fraction of organic contaminants, enabling the understanding of chemical bioavailability from the test sediments.

Sediments from 11 of the 12 the Clinch River watershed sites (the sediment traps from the Wallen Bend site were not recovered) and three uncontaminated control sediments comprised the 14 test treatments. Two of the control sediments, Spring River (SP, Upper Spring River Basin, Missouri, USA) and West Bearskin (WBS, from West Bearskin Lake, Minnesota, USA) have been previously characterized and are routinely used in toxicity testing with benthic macroinvertebrates (Ingersoll et al. 1998, 2008; Wang et al. 2013). The third control sediment was a commercially available, contaminant-free filter sand (SAN) (Southern Products and Silica Co., Inc. Hoffman, North Carolina, USA), which has been used in previous toxicological studies with freshwater mussels in our laboratory (Archambault et al. 2013, 2014a, 2014b).

The test was conducted in light- and temperature-controlled environmental chambers (Precision Model 818, Thermo Fisher Scientific, Marietta, Ohio, USA), held at 20°C and a light and dark cycle of 16:8, and followed guidelines for water-only toxicity testing with early life stage freshwater mussels (ASTM 2013), but modified as needed to accommodate sediment test conditions. Overlying water was renewed in each chamber every 72 h at 75% volume to avoid disturbing the sediment; minimal disturbance was achieved by using a vacuum pump to draw out overlying water, and then slowly replacing fresh water along the chamber sides with a peristaltic pump. Water quality was sampled weekly; mean conditions among all treatments were 164 mg $CaCO_3$/L alkalinity, 178 mg $CaCO_3$/L hardness, 547 µS/cm conductivity, 8.43 pH, and 7.77 mg/L dissolved oxygen (n = 60). Individual treatment means ranged 84 – 209 mg $CaCO_3$/L alkalinity, 156 – 200 mg $CaCO_3$/L hardness, 520 – 578 µS/cm conductivity, 7.72 – 8.69 pH, and 6.98 – 8.89 mg/L dissolved oxygen (n = 15). Additionally, water samples for total ammonia nitrogen (TAN) analysis were collected weekly, immediately preserved with sulfuric acid, and stored at 4°C in accordance with standard methods, until they were analyzed by a state-certified

laboratory (Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, North Carolina, USA).

Though it was understood that the volume of natural sediments should provide ample nutrition over the duration of the test (Naimo et al. 2000a, 2000b), each replicate was dosed with a commonly-used food mix as a precaution against underfeeding. A 1-L solution with a mixture of 2 mL Instant Algae® Shellfish Diet and 1 mL *Nannochloropsis* (Nanno 3600) concentrate diluted in deionized water was prepared, and approximately 5 mL was added to each replicate (administered concentrations of 25,000 and 425,000 cells/mL solution, respectively; Reed Mariculture, Campbell, California, USA) every 72 h after water chemistry samples were collected and at least 2 h before each water renewal. The sand treatment received 10 mL (administered concentrations of 50,000 and 850,000 cells/mL solution, respectively) to compensate for a lack of organic material (i.e., natural food source) in the substratum (Leonard et al. 2014; Archambault et al. 2015).

Test organisms

The early life stages of freshwater mussels are especially important in toxicity testing. Mussels are particularly susceptible to toxicants and other environmental stressors because of their unique parasitic (upon a host fish) and multi-stage life cycle, and juveniles may be especially sensitive, in part because they have less capacity to avoid exposure than adults (Cope et al. 2008). Therefore, juvenile Cumberlandian combshell mussels (*Epioblasma brevidens*) were used to test the toxicity of sediments collected from the Clinch River watershed. The Cumberlandian combshell is native to the Tennessee-Cumberland physiographic province. Having been extirpated from much of its historic range, with excessive sedimentation among the most important historic and current threats, it is listed as federally-endangered, and the Clinch

River is among the last remaining strongholds for persisting populations (USFWS 2004). Juveniles used in the test were propagated via host-fish infection (Aquatic Wildlife Conservation Center, Virginia Department of Game & Inland Fisheries, Marion, Virginia, USA) from an adult female mussel collected from the Clinch River at Clinchport, Virginia, using standard propagation and culture methods (Barnhart 2006). Test mussels arrived from the hatchery via overnight courier and were slowly acclimated from culture water to test water and to 20°C following ASTM guidelines (ASTM 2013). Following acclimation, mussels were held in an incubator and aerated until the experiment commenced. At the beginning of the exposure, the juveniles were 6 months old with a mean initial shell length of 1.91 mm (± 0.27 mm, SD).

Sediment characterization

Sediments collected from the Clinch River Basin were largely dominated by sand (mean 70.4%, range 42.5 – 92.0%). Those collected from the Cleveland and Copper Creek sites had the lowest sand content (42.5 and 54.3%, respectively), and contained a higher percentage of silt (44.5 and 37.2%, respectively) compared to other sediments (mean 21.9%). Clay content was relatively low in all collected sediments (mean 7.7%, range 4.4 – 13.0%). Total organic carbon (TOC) varied among Clinch sediments (range 2.4 – 8.5%), with no apparent spatial pattern. The Spring River and West Bearskin control sediments represented low and high TOC conditions (0.70 and 8.80%, respectively), and were both dominated by sand (75.9 and 54.0%, respectively), though West Bearskin had a substantial silt component (39.6%). Together they represented a range of conditions similar to sediments collected from the study area. The sand control was > 99% silica sand as SiO_2 and contained < 0.1% TOC (Table S1).

Contaminant analysis and quality control

Chemicals were measured in subsamples of each sediment collected before they were added to the test chambers, at end of the exposure, and in the dissolved phase in the overlying water during the test with polyethylene and universal PSDs to estimate exposure and indicate relative partitioning of organic constituents. Sediments were analyzed for metals (n = 22) and organic contaminants, including PAHs (n = 42), polychlorinated biphenyls (PCBs, n = 21), and organochlorine (n = 28) and current use (n = 47) pesticides. Of the 160 substances analyzed, about half of them are listed as priority pollutants (US EPA 2015).

Analyses of metal concentrations were performed using standard methods and approved protocols, including EPA Method 3050B (RTI International, Research Triangle Park, North Carolina, USA). The rigorous quality assurance protocol followed for metals analyses included reagent blanks, reagent blank spikes, duplicates, matrix spikes, and surrogate internal standards. Average recovery in surrogate standards was 87% (range 57 – 136%), relative percent difference (RPD) of duplicates averaged 11% (range 0 – 40%), recovery of matrix spikes averaged 97% (range 7 – 212%), reagent blanks were uncontaminated, and average recovery from reagent blank spikes was 101% (range 96 – 114%).

Organic contaminants in sediments and PSDs were analyzed using a gas chromatograph-mass spectrometer (Chemical Exposure Assessment Laboratory, North Carolina State University, Raleigh, North Carolina, USA) following methods described previously (Luellen et al. 2002, Thorsen et al. 2004, Heltsley et al. 2005, Hewitt et al. 2006, Cope et al. 2011). The rigorous quality assurance protocol followed for organics analyses included procedural blanks, polyethylene and universal PSD blanks, surrogate internal standards, and matrix spikes. All of the quality control data were within acceptable ranges and indicated a very high quality data set.

Surrogate recoveries for PCBs and organochlorine and current use pesticides for sediments and PSDs were nearly all between 85% and 105%, with the only exception being slightly higher recoveries for PCB 197 (≤ 130%).

Surrogate recoveries for sediment PAH analyses ranged from 52 to102%, with lower recoveries (52-89%) for the more volatile surrogates and higher recoveries (59-102%) for the less volatile surrogates. Duplicate analysis yielded RPDs below 10% for all analytes. Matrix spikes yielded recoveries between 78 and 104%, with RPDs below 10%. Surrogate recoveries for PSD PAH analysis ranged from 62 to 103% with little difference among the surrogates indicating equal recovery of all PAHs from the PSD samples. Duplicate analysis yielded RPDs below 10% for all analytes. Matrix spikes yielded recoveries between 73 and 104%, with RPDs below 10%. The data were not corrected for surrogate recoveries because any corrections would have been small and would not alter interpretation of the data. Procedural blanks contained very small amounts of phenanthrene (~ 1 ng in both) and C1-naphthalenes (3 ng and 0 ng). These amounts were well below the amounts measured in the samples so no blank subtraction was performed.

Estimating toxicity to mussels

At the end of the 28-d exposure, mussels were recovered by rinsing the sediment through a 2-mm sieve onto a 0.5 mm sieve and were found with the aid of a magnifying lamp. Survival of juvenile mussels was assessed by viewing them under a stereomicroscope; juveniles that exhibited foot movement outside or inside of the shell, shell movement, or a detectable heartbeat within a five-minute observation period were considered alive (ASTM 2013). Mussel length was determined for every mussel using digital stereoscope photographs. The percentage of length increase of mussels for the 28-d exposure was calculated as the difference between the mean

final and initial lengths of mussels in a replicate divided by the mean initial length in a replicate. After photographing their final length, mussels were transferred to 8% formalin prior to dry weight measurement. Preserved mussels were dried for 24 h at 60° C and dry weight was measured to the nearest 0.001 mg with a microbalance (Orion Cahn® C-35, Thermo Electron Corporation, Beverly, Massachusetts, USA) for determination of biomass (total dry weight of surviving mussels in a replicate). Mussel biomass was normalized to a baseline sample of 35 mussels (five replicates of seven mussels) collected and weighed at the beginning of the experiment.

Differences in mean survival, percent length increase, and departure from baseline biomass among treatments were determined by analysis of variance (PROC GLM; SAS version 9.4; SAS Institute, Inc., Cary, North Carolina, USA). Findings of significance ($\alpha = 0.05$) were followed by post-hoc analyses: Tukey's HSD was used to determine pairwise differences in the responses among treatments, and a Dunnett's test was used to identify treatments that were significantly different from the mean of the three control treatments (SAN, SP, WBS). Prior to the Dunnett's test, an analysis of variance was conducted to confirm that mussel responses in the control treatments were not statistically different. Tukey's test is informative of the relative differences in mussel responses to sediment exposure among sites in the Clinch River, and is important to understanding the recent trends in mussel decline at some sites compared to the stability of mussel populations at other sites. The Dunnett's test provides a measure of potential toxicity of a river sediment from a particular treatment site when compared to uncontaminated control sediments. Because selecting the standard $\alpha = 0.05$ may be somewhat arbitrary in tests of statistical significance, and because biologically meaningful differences may not always be

captured at this level, Dunnett and Tukey post-hoc differences at the $\alpha = 0.10$ level are also reported.

Characterizing sediment toxicity

Sediment quality guidelines provide probable effect concentrations (PEC) as an indicator of toxicity to freshwater organisms for nine of the 22 metals analyzed (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) (Persaud et al. 1993; MacDonald et al. 2000). Individual PEC quotients (PECQ) were calculated for each metal in each sediment sample as a measure of toxicity by dividing the dry-weight concentration of the metal by its respective PEC, and then deriving the average PECQ for each sample. An average PECQ of > 1.0 is considered toxic to aquatic life; however, these sediment quality guidelines were derived from datasets that do not include toxicity to unionoid mussels, and recent research by others has found sediments with values < 0.4 to be toxic to mussels, indicating that PECs may not be protective of mussels (Wang et al. 2013). Our results are considered in this updated context.

Toxicity of PAH concentrations was evaluated using the most up-to-date EPA methods for evaluating toxicity in sediments, based on a mechanistic approach that incorporates both the additivity of PAH toxicity and the reduction in PAH bioavailability caused by TOC in the sediment (US EPA 2003). Raw individual PAH concentrations in each sediment sample were normalized to the TOC fraction and then divided by the respective acute or chronic EPA Potency Divisors (available for 34 PAHs; US EPA 2003) to calculate the equilibrium-partitioning sediment benchmark toxic unit (ESBTU). The individual PAH ESBTUs were totaled; the resulting sum ESBTU ($\sum$ESBTU, hereafter simply referred to as TU) is a measure of Total PAH toxicity. Values ≥ 1.0 indicate the possibility of harm to aquatic life. Published PECs for PAHs in sediment quality guidelines are considered a more outdated measure of toxicity because

bioavailability is unaccounted for and they are not mechanistically based. However, we also report our results in such a context with the Total PAH PEC because many researchers are familiar with such thresholds, and to emphasize the potential lack of protection under such guidelines for freshwater mussels, as has been reported by others (Wang et al. 2013).

Measured TAN concentrations were evaluated in the context of the recently-updated Water Quality Criteria for protection of aquatic life (US EPA 2013). The new criteria document now includes toxicity data for unionoid mussels and other freshwater mollusks, which are among the taxa most sensitive to ammonia and whose inclusion drives the criteria substantially lower than previously published iterations. Our TAN results are reported and discussed for comparison to current Water Quality Criteria, which include acute and chronic limits for concentrations allowed in surface waters. The acute criterion is defined as a 1-hr average concentration at a given temperature and pH that should not be exceeded more than once every three years (US EPA 2013). The chronic criterion is defined as a 30-day rolling average that should not be exceeded more than once every three years on average; further, the highest 4-day average within a 30-day period should not exceed 2.5X the chronic criterion more than once in three years (US EPA 2013). For example, the acute and chronic criteria under conditions of 20°C and 7.0 pH are 17 and 1.9 mg TAN/L, respectively, while the 4-day limit is 4.8 mg TAN/L (2.5 * the chronic criterion) under the same conditions.

To gain a better understanding in the driving influences behind significant effects of sediment treatments, we conducted correlation analyses between significant responses and all parameters analyzed – sediment concentrations of metals and organic contaminants, water quality, and sediment characteristics (PROC CORR, SAS version 9.4). Pearson product-moment correlations were used where relationships between a parameter and response were linear, and

Spearman rank-order correlation coefficients were used if a relationship was nonlinear or there were outliers. Significant correlations were used to populate explanatory models for a given response, thus producing quantitative estimates of effects parameters (PROC GLMSELECT, SAS version 9.4). The most plausible, parsimonious models explaining survival and biomass were selected from all possible models using Akaike's Information Criterion adjusted for low sample sizes (AIC_C; Burnham and Anderson 2002).

Results

Mussel toxicity

Recovery of mussels (n = 276) from the experimental chambers was 94%. Mean survival experiment-wide was 82.6% and ranged from 33.3% (Guest River) to 100% in three of the treatments, including one control (Spring River). Sediment treatment had a significant effect on survival of juvenile mussels ($p < 0.01$; Figure 2). Compared to controls, mussels exposed to Guest River sediments had significantly lower survival ($p < 0.05$), and Tukey's pairwise comparisons among all treatments revealed differences between survival in the Guest River treatment versus Dumps Creek and Pendleton. Using an $\alpha = 0.10$, survival in Copper Creek sediments was also lower than in control sediments, survival in Guest River was lower than in all treatments except Carterton, Simones, and Copper Creek (Figure 2).

Mean biomass of mussels in the baseline samples was 4.661 mg (± 0.219 mg, SE). Mean departures from the baseline at the end of the exposure ranged from -46.8% (Copper Creek) to +47.3% (Indian Creek) and was significantly affected by sediment treatment ($p = 0.02$). Mussels in all three control sediments gained biomass (9.5 – 40.2% increase), as did mussels in the Carterton, Pendleton, and Clinchport mainstem sediments and Dumps and Indian Creek tributary

sediments (15.4 – 47.3%; Figure 3). Mussels in six treatments lost biomass during the test, including the greatest losses occurring in the Copper Creek and Guest River treatments (46.8 and 41.5% biomass loss, respectively). Those in Artrip, Cleveland, and Simones sediments were negative, but confidence limits crossed the line of no change (Figure 3). Mussels in Guest River and Copper Creek sediments had significantly less biomass when compared to control mussels, and, mussels in those sites also had significantly less biomass than those exposed to Indian Creek sediments ($p < 0.10$; Figure 3). Mussels in the Copper Creek sediment also had significantly less biomass than those in the Spring River treatment ($p < 0.10$). There were no differences in biomass among sites in post-hoc analyses when using $\alpha = 0.05$. Percent length increase was not significantly affected by sediment treatment ($p = 0.37$). Length increase overall was minimal (mean increase = 3.3%, range 1.5 – 6.6%) and varied within and among treatments.

Overlying water ammonia

Median ammonia concentrations in overlying water ranged 0.01 – 0.32 mg TAN/L in control treatments and 0.47 – 1.58 mg TAN/L in Clinch River Basin treatments. The median was selected as the most appropriate measure of central tendency of concentrations among weeks, in part, because week-1 ammonia concentrations were high (outliers) compared to measurements at nearly every other time interval. Moreover, the median better reflected the long-term trend in exposure than did the mean. For context of the results, the 1-hour (acute), 4-day, and 30-day (chronic) water quality criteria at the test temperature (20°C) and average test pH of 8.43 are 1.8, 1.0, and 0.41 mg TAN/L, respectively (US EPA 2013; Figure 4).

Ammonia concentrations in overlying water were extremely high in several treatments during week 1 of the experiment (mean 4.16 mg TAN/L and range 0.71 – 6.26 mg TAN/L in Clinch sediments; range <0.01 – 1.89 mg TAN/L in controls) when the duration of contact with

sediments was the longest (day -3 through day 3), followed by a precipitous decline in most treatments at the week 2 sampling point after two water renewals (day 10). However, ammonia remained high in the Guest River and Copper Creek treatments (3.13 and 2.0 mg TAN/L, respectively) compared to others (range 0.02 – 1.24 mg TAN/L in all other Clinch sediments, 0.01 – 0.62 mg TAN/L in controls) (Figure 4, Table S2). Week 1 ammonia concentrations exceeded the 1-hr acute ammonia water quality criterion values for the protection of aquatic life (range 1.0 – 1.8 mg/L at respective mean treatment pHs; US EPA 2013) in every Clinch treatment except Dumps Creek. In week 2, ammonia in only the Guest River and Copper Creek treatments remained above the respective 1-hr acute criteria; ammonia in the Indian Creek, Artrip, Cleveland and Clinchport treatments was above the 4-day limit (range 0.63 – 1.03 mg/L at test conditions). Ammonia in all other treatments was below the respective 30-day chronic criterion (range 0.25 – 0.41 mg/L). In weeks 3 and 4, ammonia concentrations were < 0.1 mg TAN/L and below all ammonia criteria values for all treatments.

Sediment contaminants

Metals. A majority of the 22 metals selected for analysis were detected in most treatments, with the exception of Cd and Se, which were not detected in any treatment, and Hg and Mo, which were found at low concentrations in only one and two treatments, respectively. Mean metal PECQs for Clinch River Basin sediment treatments ranged from 0.10 to 0.17; treatments with the highest values included the Dumps Creek (0.17), Copper Creek (0.16), and Guest River (0.15) tributaries. Spring River and West Bearskin control sediments had mean PECQs of 0.08 and 0.14, respectively. A mean PECQ for the sand control was not calculated because concentrations were below detection limits for eight of the nine metals for which PECs are available; only Zn was present, but at a very low concentration (1.20 µg/g sediment,

compared to the Zn PEC of 459 µg/g). Individual PECQs for As, Cr, Cu, Fe, Mn, Ni, Pb, and Zn among treatments ranged from 0.03 to 0.57, with a mean value of 0.13 (Table 1). Average individual PECQs among treatments for As, Cr, Cu, Pb, and Zn were each < 0.1. PECQs for Cd were not calculated because concentrations were below the detection limit (0.25 µg/g sediment) for all treatments. Mn had the highest PECQ within each Clinch sediment treatment compared to other metals, averaging 0.34 (range 0.18 – 0.57) among treatments, which is more than double the control sediment values (SPR = 0.13, WBS = 0.12). Mn concentrations ranged 197 – 630 µg/g sediment (Figure 5). The individual PECQ average for Fe (0.20) was also relatively high compared to other metals, though it fell between the control sediment values (SPR = 0.10, WBS = 0.30). Notable results among metals for which no toxicity benchmarks are available include K and Mg; mean concentrations among Clinch treatments (445 and 1298 µg/g, respectively) were well above control means (267 and 776 µg/g, respectively) for both (Table S3).

Differences between pre- and post-test metal concentrations were small in most cases. The mean absolute difference in total metal concentrations was 7.6% (absolute median 6.8%, absolute range 1.7 – 16.4%) among Clinch sediments. Four post-test samples had slightly higher average metal concentrations than pre-test samples, indicating that the overall differences merely represent within-sample variation, and that no appreciable changes can be attributed to uptake by mussels or loss to the dissolved fraction in overlying water. Further, this variation resulted in no appreciable change in mean metal PECQ (absolute differences ranged 0 – 0.02).

Comparison of post-test sediment samples from replicates with mussels to the replicate with no mussels had similar results. The mean absolute difference in total metal concentrations was 6.1% (absolute median 4.5%, absolute range 0.5 – 16.5%) among Clinch sediments. In four treatments, the sample that contained mussels had slightly higher total metals than the sample

that had no mussels, indicating again that the small differences noted are a product of variation within a given sediment. Moreover, we cannot distinguish between loss to the soluble fraction of overlying water and uptake by mussels.

Organics. None of the 47 current use pesticides or 21 PCBs were detected in any of the sediment samples. Trans-nonachlor was the only organochlorine pesticide detected and was found only in the Guest River sediment at a very low concentration of 2.56 ng/g (dry weight sediment), indicating a only a very small residue of degraded chlordane.

In contrast, PAHs were found in every sediment sample. Concentrations of Total PAH (sum of the 42 PAHs analyzed) ranged from 364 ng/g in the Spring River control sediment to 54,129 ng/g at Carterton (Figure 6A; Table S4). Concentrations were above 1000 ng/g in all Clinch River sediments indicating widespread input of PAHs to the system. The West Bearskin control concentration of 1484 ng/g was mostly perylene, which is common in sediments with high organic content and is associated with non-anthropogenic sources such as woody vegetation (Gricea et al. 2009) and diatoms (Venkatesan 1988). With the exception of Pendleton (5189 ng/g), all sediments from the mussel zone of decline had Total PAH concentrations > 6500 ng/g. These elevated PAH concentrations in Clinch River treatments exceeded consensus-based PEC values (MacDonald et al. 2000) for a few individual PAHs in a few treatments, but only the Carterton treatment exceeded the PEC (22,800 ng/g) for Total PAH. PECQs for Total PAH by treatment ranged from ≤ 0.07 in controls to 2.37 in the Carterton sediment. Clinchport, Guest River, and Dumps Creek sediments had notably elevated PECQs (0.65, 0.52, and 0.32, respectively). The more updated ∑ESBTU approach resulted in acute TU values ranging < 0.01 – 0.37 and chronic TU values ranging 0.02 – 1.51. Only the Carterton site exceed a chronic TU of 1.0, but several sites were clearly elevated above the others (Figure 6B). These included

Pendleton (0.21) and Guest River (0.27), and especially Dumps Creek and Clinchport, where TU values for both were above 0.40.

Most sediments were dominated by petrogenic PAHs (i.e., fossil fuel-sourced including most of the PAH from unburned coal), accounting for 65 – 85% of Total PAH compared to the pyrogenic portion (i.e., sourced from combustion/burning of fossil fuels). Petrogenic and pyrogenic PAHs were more evenly represented in Clinchport sediment (55 and 45%, respectively). Contrary to most other treatments, pyrogenic PAHs were the dominant source in Carterton and Copper Creek sediments, accounting for 71 and 64% (respectively) of all PAHs in those sediments, and petrogenic PAHs contributing ≤ 36%.

Differences between pre- and post-test PAH concentrations were negligible. The mean absolute difference in Total PAH concentrations was 7.0% (absolute median 5.3%, absolute range 0.3 – 20.7%). Four post-test samples had slightly higher Total PAH concentrations than pre-test samples, indicating that the overall differences merely represent within-sample variation, and that no appreciable changes can be attributed to uptake by mussels or loss to the dissolved fraction in overlying water. Further, this variation resulted in no appreciable change in TU (all acute TU change = 0; chronic TU change = 0 for all sites except Dumps Creek, where the difference was 0.1).

Comparison of post-test sediment samples from replicates with mussels to the replicate with no mussels had similar results. The mean absolute difference in Total PAH concentrations was 9.4% (absolute median 6.8%, absolute range 2 – 20.2%). In seven treatments, the sample from replicates that contained mussels had a slightly higher Total PAH than the sample that had no mussels, indicating again that the small differences noted are a product of variation within a

given sediment. Moreover, we cannot distinguish between loss to the soluble fraction of overlying water and uptake by mussels.

Passive sampling devices

Dissolved fraction PAHs were detected in overlying water by PSDs in all treatments. There was a much smaller range of PAH concentrations in the PSDs compared to those in sediments, with Total PAH ranging from ~1550 ng/L to 3350 ng/L. Corresponding chronic TU values also had a smaller range (0.15 – 0.46). Thus, none of the samples exceeded the toxicity benchmark of 1.0, but all were elevated above background. The relative abundance of PAHs in the PSDs was rather consistent among exposures, with petrogenic PAHs comprising about 65-80% of the PAHs, and pyrogenic PAHs only 20-35%. Naphthalene was not detected in any of the PSDs, indicating possible loss of more volatile PAHs from the water, and that the concentrations of other more volatile PAHs may be artificially low, as well. Similar to results from the sediment analyses, few other organic contaminants were detected in the dissolved fraction. No PCBs or current use pesticides were detected, and there were only two very low detections of chlordane-related organochlorine pesticides in the polyethylene PSDs. Moreover, the two PSD types provided a consistent indication of no or very low exposure to PCBs and organochlorine and current use pesticides from the sediments at all sites.

Correlation analysis and explanatory statistical models

Only Mn and Co were significantly correlated with mussel survival and biomass, as was median ammonia concentration with survival, and TOC with biomass (Figure 7, Table 2). No other metals, water quality parameters, or sediment characteristics were correlated with biotic endpoints. Further, there were no significant correlations between biotic endpoints and TOC-normalized sediment concentrations of individual PAHs or Total PAH acute or chronic TUs.

Because Mn and Co covaried perfectly and Mn was present in much higher concentrations than Co, Mn was used as the candidate parameter in quantitative model selections for both survival and biomass. In addition to the other correlated variables, Total PAH chronic TU was included as a candidate parameter, despite a lack of specific correlation, to represent organics in the model selection process because of overall PAH abundance and detection among treatments.

In quantifying the effects of variables on endpoints using model selection, we restricted the models to main effects only. Attempts to quantify interactive effects with our dataset and level of replication resulted in several equally-plausible models and were uninformative (i.e., models with interactive terms were equivalent to those without).

Two linear regression models explaining mussel survival were equally parsimonious (essentially equivalent AIC_C and model weight (w_i); Table 3). The first model ($F_{1,37} = 23.29$, $p < 0.01$; $r^2 = 0.39$) included only ammonia and estimated that survival was reduced by approximately 33% with every 1 mg/L increase in median TAN. The second equally plausible model explaining survival ($F_{2,36} = 13.20$, $p < 0.01$; $r^2 = 0.42$) estimated negative effects of both ammonia and Mn, though the Mn term was not significant ($p = 0.14$). Survival was reduced by approximately 26% per unit increase in median TAN at a given Mn concentration (i.e., if Mn was held constant). For a given ammonia concentration, Mn reduced survival by 0.04% for every 1 µg/g increase; on a more relevant scale, a 100 µg/g increase in Mn resulted in a 4% reduction in survival (Table 3). In other analyses (not reported), when mean ammonia was considered instead of the median, Mn was a highly significant parameter ($p < 0.01$) along with ammonia in the most parsimonious model.

The most parsimonious model explaining the observed trends and significant effects of sediments on biomass included negative effects of Mn and TOC ($F_{2,36} = 11.44$, $p < 0.01$; $r^2 =$

0.39). Mn reduced biomass by 14% per 100 µg/g increase in concentration at a given TOC level (or 0.14% per 1 µg/g increase as shown in Table 3). For every 1% increase in TOC, biomass was reduced by 6.9%. There was substantially less support for any other models explaining biomass in the selection (Table 3).

Discussion

Sediments used in toxicity tests with aquatic organisms are typically collected by more traditional sampling methods, such as grab samples of bed sediments in depositional areas. While sediment traps are regularly used in studies of erosion/sediment transport and particle settlement, plankton settlement, and even collection of sediments for contaminant analysis (Bartsch et al. 1996), we present here the first application of such traps (of which we are aware) in testing the toxicity of collected suspended sediments on native stream species. This technique clearly allowed for the evaluation of the toxicity of the sediment load – a poorly studied ecological compartment – in this study. Our study also adds to the growing body of research on the toxicity of sediments to native freshwater mussels and reinforces the need for mussel sediment testing guidelines, in addition to the existing water-only standard method for mussels (ASTM 2013) and the standard methods for evaluating sediment-associated contaminants with other freshwater invertebrates (ASTM 2010).

Mussel survival and biomass were detrimentally affected by ammonia and Mn in the exposures to sediments from the Clinch River watershed, as demonstrated by quantitative explanatory statistical models. The most drastic negative effects on both endpoints were observed in the sediments from the Guest River and Copper Creek tributaries, which had the greatest concentrations of both contaminants, coupled with the poorest survival and biomass.

The mechanism behind the correlation and modeled effects of TOC on mussel biomass are unclear, but the finding is consistent with recent research that found the carbon content of Clinch River bed sediments was negatively correlated with several mussel health metrics (Johnson et al. 2014).

Though Guest River and Copper Creek were the most toxic sediments in our investigation, the two watersheds have widely different land uses (Locke et al. 2006, Johnson et al. 2014; Figure 8), and Copper Creek has been considered as a candidate site for aquatic conservation (Hanlon et al. 2009) because it is one of the few tributaries in the Clinch River Basin largely unaffected by coal and natural gas mining. Despite evidence that unmined tributaries of the Valley and Ridge physiographic landform help dilute high concentrations of ions delivered from tributaries in the mined watersheds of the Appalachian Plateau (e.g., Guest River; Johnson et al. 2014), unmined tributary watersheds like Copper Creek may be sourcing other problematic pollutants (i.e., ammonia) to the Clinch River via its sediment load.

Ammonia. Several studies report acute and chronic lethal and sublethal ammonia concentrations for unionid mussels of < 1 mg TAN/L (Newton et al. 2003, Newton and Bartsch 2007, Wang et al. 2007, 2011), which is considerably lower than some concentrations that we observed in this study. While it can be argued that juveniles may pedal feed below the surface (Cope et al. 2008) and thus may be less exposed to surface/overlying water, adult mussels and juveniles as small as 3 mm have been shown to siphon at the surface or sediment/water interface (Wang et al. 2013, Archambault et al. 2014a, 2014b). Given that the overlying water concentrations of TAN were so high, it is possible that pore-water ammonia could be higher. However, toxicity is dependent on subsurface temperature and pH (US EPA 2013), both of which may be substantially lower just below the sediment surface, thus reducing toxicity

(Newton and Bartsch 2007). At least one study of bed sediments in a river found that pore water ammonia concentrations were significantly higher than those found in surface water samples, and that sediment pore water ammonia may also be temporally more variable, having much more than surface waters in warmer months when higher temperatures render it more toxic (Frazier et al. 1996).

Though surface water ammonia concentrations in the Clinch River Basin have steadily declined over the last few decades due to improving wastewater treatment practices (Johnson et al. 2014, Price et al. 2014), our findings indicate that the sediment load, especially in the Guest River and Copper Creek, are supplying an excessive amount of ammonia that could be problematic for mussels and other benthic organisms during periods of higher temperature and low flows (i.e., less flushing), such as summer, and especially drought conditions. Sediments from other sites within the mainstem of the Clinch River, including those upstream of the mussel zone of decline, also had high ammonia concentrations early in the test (i.e., weeks 1 and 2). However, ammonia in these treatments attenuated to lower concentrations much more quickly, indicating those sediments were storing comparatively less.

Even in the absence of posing an immediate threat (i.e., in higher flow/flushing and lower temperature/pH conditions), we have identified a source of ammonia contamination via the sediment load entering the Clinch River. While ammonia from human-related effluents may have been substantially rectified, other sources, such as livestock/agricultural runoff and erosion remain an issue. Nearly 41% of land use in the Copper Creek watershed is classified as hay/pasture, a pattern that has been consistent since at least 2001 (S. Alexander, US Fish and Wildlife Service, personal communication), and likely going back much farther. Though more than half of the Copper Creek watershed is forested, pastures line the banks. Livestock is

abundant, and cattle often have direct access to the stream (Hanlon et al. 2009), contributing directly to ammonia (via urine/feces elimination) and erosion/sedimentation. Poor agricultural practices coupled with increased removal of riparian vegetation have been contributing factors to water quality degradation in the Copper Creek watershed since the early 1990s; an analysis of riparian areas indicated that nearly half of the stream had either no riparian vegetation or only a single row of trees (Hanlon et al. 1999), leaving waters unprotected from siltation and nutrient runoff. At the conditions that we measured during summer 2014 sampling visits to the study sites for a companion project, the mean pH was 8.2 and upper temperatures exceeded 24°C), which would equate to acute and chronic ammonia criteria similar to those for the sediment test described here. Reduction of ammonia sourcing via improved land management is imperative because ammonia sourced from the sediment load could lead to exceedances of the protective Water Quality Criteria in the riverscape, putting mussels and other sensitive taxa at risk.

Metals. Mn is an essential nutrient in most organisms. In unionids, it functions in protection from oxidative stress by removing free radicals, but may also accumulate in excessive concentrations via the same uptake pathway as calcium, leaving mussels unable to efficiently excrete the excess (Campanella et al. 2005). After just 28 days of exposure, Mn and Co concentrations were the only sediment metal parameters that correlated significantly with mussel survival and biomass, and explanatory models indicated Mn had a significant effect on biomass, especially. Mn and Co are among the metals most likely to leach from rejected spoil rock in coal mining operations (Silva et al 2011). Johnson et al. (2014) reported that water column concentrations of Mn and Fe in the Clinch River were significantly different between sites with high-quality and low-quality mussel assemblages, and both were negatively related to mussel health metrics such as recruitment and number of imperiled species. Most coal-mining and,

therefore, spoil rock sources of Mn are limited to the Appalachian Plateaus on the northern side of the Clinch River basin (Zipper et al. 2014). While Mn contamination in the Guest River is clearly related to spoil rock from massive surface coal-mining and valley fill operations upstream, there is no mining in the Copper Creek watershed (Figure 8), and the source of elevated Mn there is both disconcerting and unclear at this time.

While mean metal PECQs for each treatment were well below the established toxicity benchmark of 1.0 for sediment quality, other investigators have found sediments with mean PECQs < 0.4 to be toxic to freshwater mussels, including some with PEC values in the same range as our results (Wang et al. 2013). As previously stated, the toxicity benchmarks were established using toxicity data primarily from other classes of invertebrates (e.g., midges and amphipods; MacDonald et al. 2000). Our results are consistent with findings by Wang et al. (2013) and further substantiate evidence that established metal PECQs may not be representative of toxicity to freshwater mussels. Moreover, researchers have begun recognizing results presented by Wang et al. (2013) as more reliable benchmarks of mussel toxicity than the older guidelines (Johnson et al. 2014, Price et al. 2014).

Organics. The prevalence of agricultural lands in the Clinch River watershed coupled with our findings of virtually absent organochlorine and current use pesticides is a promising signal that they are properly applied and of little concern, although cultivated cropland comprises only a small percentage of the agrarian proportion of land uses (S. Alexander, US Fish and Wildlife Service, personal communication). Also, collection of the sediment load during spring when the majority of pesticides are likely applied, as opposed to our late summer/autumn collection, would be prudent to further support such a conclusion. PCBs are also of apparent little concern based on our findings. Further, sediment load concentrations of PCBs and

pesticides here are consistent with low concentrations or absence of these chemicals in river bed sediments, water column, and mussel tissue residues in a companion study of other ecological compartments (Bergeron et al. 2015).

The sediment at the Carterton site had the greatest PAH content by far, compared to other Clinch River and tributary sediments. The prevalence of pyrogenic PAHs at Carterton is likely due to its close proximity and downstream of a coal-fired power plant along the Clinch River at Carbo (Virginia, USA), which serves as a perennial PAH source. The PAH signature at this site may change in the future because the Carbo plant is being converted to burn natural gas, with plans to be operational in 2016 (American Electric Power 2015). The Guest River and Dumps Creek tributaries clearly are also important PAH sources. The Guest River had Total PAH concentrations higher than most other sediments we tested. While Dumps Creek had absolute PAH concentrations that were substantially lower than those in Carterton, Clinchport, and Guest River, the individual PAHs present in the Dumps Creek sample were toxic enough that it ranks in the top three PAH chronic TU scores, surpassing Guest River.

Despite there being no statistical link to the influence of PAHs on mussel survival and biomass in our 28-day experiment, our analyses demonstrate that the sediment load contributes substantially to the influx of PAHs into the Clinch River Basin. Considering the very high PAH concentration and chronic TU at Carterton, and elevated TUs at Clinchport, Dumps Creek, and other sites within the mussel zone of decline, long-term exposure to these PAHs may affect mussels and perhaps other aquatic life. More subtle effects of PAH toxicity and that of their metabolites may take longer than 28-d to manifest via survival and biomass endpoints, or might be more obvious in a biochemical endpoint (e.g., upregulated biomarker enzymes, differential tissue concentrations; Newton and Cope 2007) that could not be addressed in this study with

juvenile mussels. Such effects may impact immunity, reproduction, or other processes that can lead to long-term population-level declines like those already reported in the Clinch River. Moreover, mixtures of PAHs with other contaminants may result in synergistic harm to mussels and other organisms. A recent review of the co-toxic effects of metal-PAH mixtures concluded that synergistic impacts were more likely than additive effects, and that the current paradigm of environmental risk assessment is ill equipped to consider such interactions (Gautier et al. 2014).

Implications/Conclusions

Mussel survival and biomass were affected most drastically by ammonia and Mn sourced from the sediment load collected with sediment traps from the Clinch River Basin waters, and especially sediments from the tributaries, Guest River and Copper Creek. Long-term exposures to such conditions at these sites could be problematic for juvenile and/or adult mussels and other freshwater fauna. Additionally, full chronic exposures (over months or years, compared to our 28-day test) could allow other contaminants, especially the prevalent PAHs, to adversely affect mussel biomass and survival. More importantly, such contaminants are already known to alter aquatic fauna at sub-cellular scales (e.g., carcinogenesis, teratogenesis; Newton and Cope 2007, Prochazka et al. 2012), and thus, may impact immunity and fecundity in mussels, in addition to the more coarse-scale endpoints examined here. This sediment-load burden of PAHs, ammonia, Mn, and elevated concentrations of other metals provides evidence that many sites within the basin are being regularly renewed with such contaminants. Evaluating the ramifications of toxicant mixtures is an emerging science, largely because such studies are complex and difficult to accomplish, but current knowledge indicates negative synergistic effects are the most likely outcome (Gautier et al. 2014). Therefore, future laboratory studies similar to this one may

consider even greater robustness in their designs (e.g., more replication, more frequent assessment of endpoints (may require destructive samples)) to quantify interactive effects and evaluate sub-cellular endpoints. Finally, the sediment load is an important source of contaminant stressors that should not be ignored, and our results here can be applied to watershed management and faunal conservation in the Clinch River and other systems in context with knowledge of whole-system contaminant cycling and function. Wider implementation of established best management practices, such as restricting direct livestock access to streams in favor of watering stations and restoration of sufficiently-wide riparian buffers, could substantially reduce sedimentation, thereby reducing the contribution of contaminants from the sediment load and improving the overall health of the Clinch River ecosystem.

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Figure legends

Figure 1. Map of study area and sediment collection sites in the Clinch River watershed. There were eight mainstem (dark circles) and four tributary sites (white triangles). Zone of documented mussel decline is denoted within black bars. Indian Creek, Artrip, and Cleveland are located upstream of the mussel zone of decline; Dumps Creek, Carterton, Guest River, Simones, Pendleton, and Clinchport are within the zone of decline; Copper Creek, Horton Ford, and Wallen Bend are downstream of the zone of decline.

Figure 2. Mean survival (± SE) of freshwater mussels in a 28-day test exposed to control (white bars) and Clinch River sediments (grey bars = mainstem, hatched bars = tributary, arranged in order from upstream to downstream). Treatment (site) names and abbreviations are as follows: Spring River, SP; Sand, SAN; West Bearskin, WBS; Indian Creek, IN; Artrip, ART; Cleveland, CLE; Dumps Creek, DC; Carterton, CAR; Guest River, GR; Simones, SIM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Sites in the documented zone of mussel decline are denoted by the surrounding box. There was a significant effect of sediment treatment on survival (ANOVA $p < 0.01$). A Dunnett's post-hoc test revealed significantly lower survival compared to control in the Guest River ($p < 0.05$, filled star) and Copper Creek ($p < 0.10$, empty star) treatments. In Tukey's pairwise comparisons, survival in Guest River was significantly lower than two controls and the Pendleton treatment at the $\alpha = 0.05$ level (capital letters), and lower than most treatments at the $\alpha = 0.10$ level (lowercase letters). Unlabeled bars were not different (i.e., AB).

Figure 3. Mean change in biomass (± SE) of freshwater mussels in a 28-day test exposed to control (white bars) and Clinch River sediments (grey bars = mainstem, hatched bars = tributary, arranged in order from upstream to downstream) compared to initial baseline biomass samples. Treatment (site) names and abbreviations are as follows: Spring River, SP; Sand, SAN; West Bearskin, WBS; Indian Creek, IN; Artrip, ART; Cleveland, CLE; Dumps Creek, DC; Carterton, CAR; Guest River, GR; Simones, SIM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Sites in the documented zone of mussel decline are denoted by the surrounding box. Treatments with bars above 0 gained biomass and those below 0 lost biomass. There was a significant effect of sediment treatment on biomass (ANOVA $p = 0.02$). A Dunnett's post-hoc test revealed significantly lower biomass compared to the control in the Guest River and Copper Creek treatments ($p < 0.10$, empty stars). In Tukey's pairwise comparisons, biomass in Guest River sediment was significantly lower than in Indian Creek sediment, and biomass in Copper Creek sediment was lower than in Indian Creek sediment and a control ($p < 0.10$, lowercase letters). Unlabeled bars were not different (i.e., abc) and there were no pairwise differences at the $\alpha = 0.05$ level.

Figure 4. Measured total ammonia nitrogen (TAN) concentrations in overlying water samples (one composite sample/treatment) at week 1 (day 3, black bars) and week 2 (day 10, grey bars) during the 28-day sediment test with freshwater mussels. Horizontal lines indicate the 1-hr, 4-day, and 30-day criterion (1.8, 1.0, and 0.41 mg TAN/L, respectively) at the test temperature (20°C) and overall mean pH of 8.4. While individual treatment pH varied slightly, the trend of criterion exceedances matches. Treatment (site) names and abbreviations are as follows: Spring River, SP; Sand, SAN; West Bearskin, WBS; Indian Creek, IN; Artrip, ART; Cleveland, CLE;

Dumps Creek, DC; Carterton, CAR; Guest River, GR; Simones, SIM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Sites in the documented zone of mussel decline are denoted by the surrounding box.

Note: The only treatment for which the criteria and exceedances shown do not apply is the West Bearskin control (WBS), which has much more lenient criteria due to the comparatively lower pH (7.7). The 1-hr, 4-day, and 30-day criteria at 20°C for WBS are 6.7, 2.8, and 1.1 mg/L, respectively.

Figure 5. Measured manganese (Mn) concentrations (A) and calculated probable effect concentration (PEC) quotients (B) in control (white bars) and Clinch River watershed sediments (grey bars = mainstem, hatched bars = tributary, arranged in order from upstream to downstream) at the beginning of the 28-day sediment test with freshwater mussels. Treatment (site) names and abbreviations are as follows: Spring River, SP; Sand, SAN; West Bearskin, WBS; Indian Creek, IN; Artrip, ART; Cleveland, CLE; Dumps Creek, DC; Carterton, CAR; Guest River, GR; Simones, SIM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Sites in the documented zone of mussel decline are denoted by the surrounding box.

Figure 6. Total concentration of polycyclic aromatic hydrocarbons (PAH; sum of all 42 measured PAHs) from sediment samples taken at the beginning of the 28-day test with freshwater mussels (A), and corresponding sum equilibrium-partitioning sediment benchmarks toxic units for chronic toxicity (Chronic ∑ESBTUs) (B), with a horizontal line denoting the accepted standard of toxicity for benthic organisms at 1.0 in control (white bars) and Clinch River watershed sediments (grey bars = mainstem, hatched bars = tributary, arranged in order from upstream to downstream). Treatment (site) names and abbreviations are as follows: Spring River, SP; Sand, SAN; West Bearskin, WBS; Indian Creek, IN; Artrip, ART; Cleveland, CLE; Dumps Creek, DC; Carterton, CAR; Guest River, GR; Simones, SIM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Sites in the documented zone of mussel decline are denoted by the surrounding box.

Figure 7. Linear relationships between significantly correlated treatment constituents and mean mussel endpoints from the 28-day sediment test with freshwater mussels: (A) Manganese (Mn) vs. survival, (B) Mn vs. biomass, (C) total ammonia nitrogen (TAN) vs. survival, and (D) carbon (sediment total organic percent) vs. biomass.

Figure 8. Land cover/land use categories in the Guest River (A) and Copper Creek (B) sub-watersheds of the Clinch River Basin. Prominent colors represent the following land use/land cover categories: greens = forested; reds = developed; yellow = hay/pasture; purple outline = surface mines.

Figure 1.

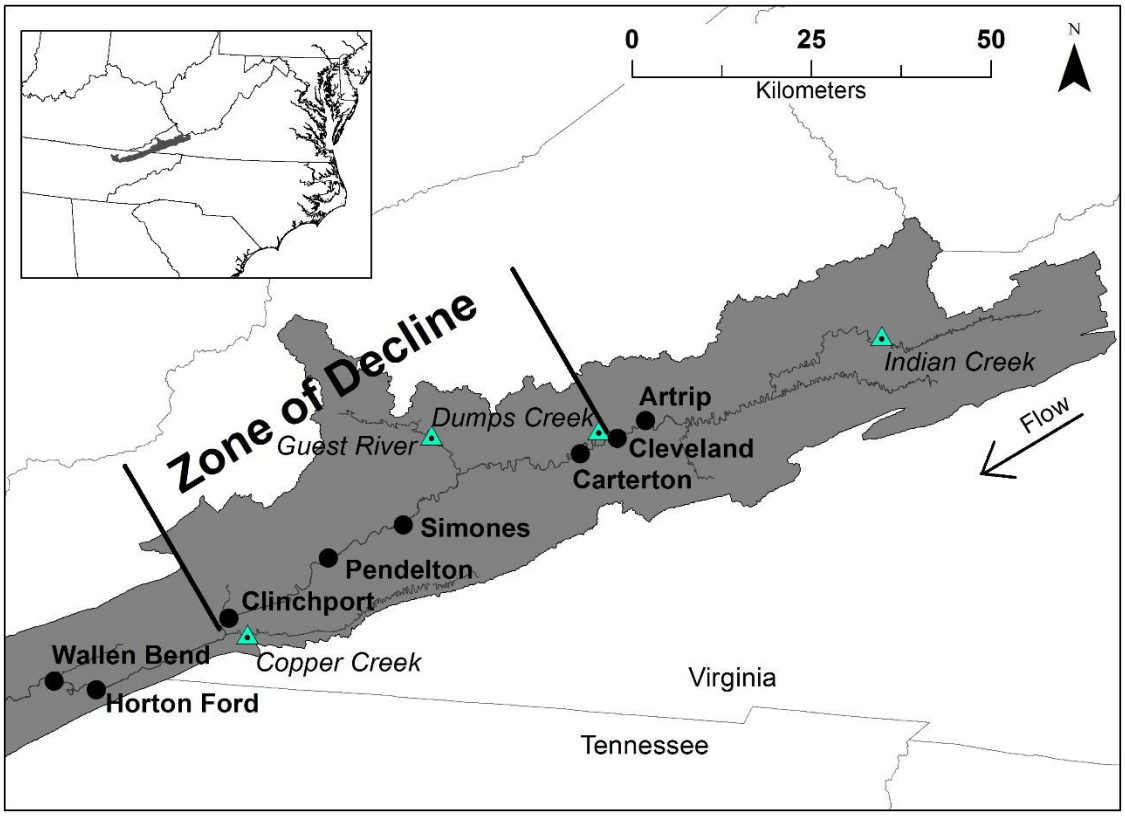

0
25
50
Kilometers
N
Zone of Decline
Indian Creek
Artrip
Dumps Creek
Guest River
Cleveland
Carterton
Flow
Simones
Pendelton
Clinchport
Wallen Bend
Copper Creek
Virginia
Horton Ford
Tennessee

Figure 2.

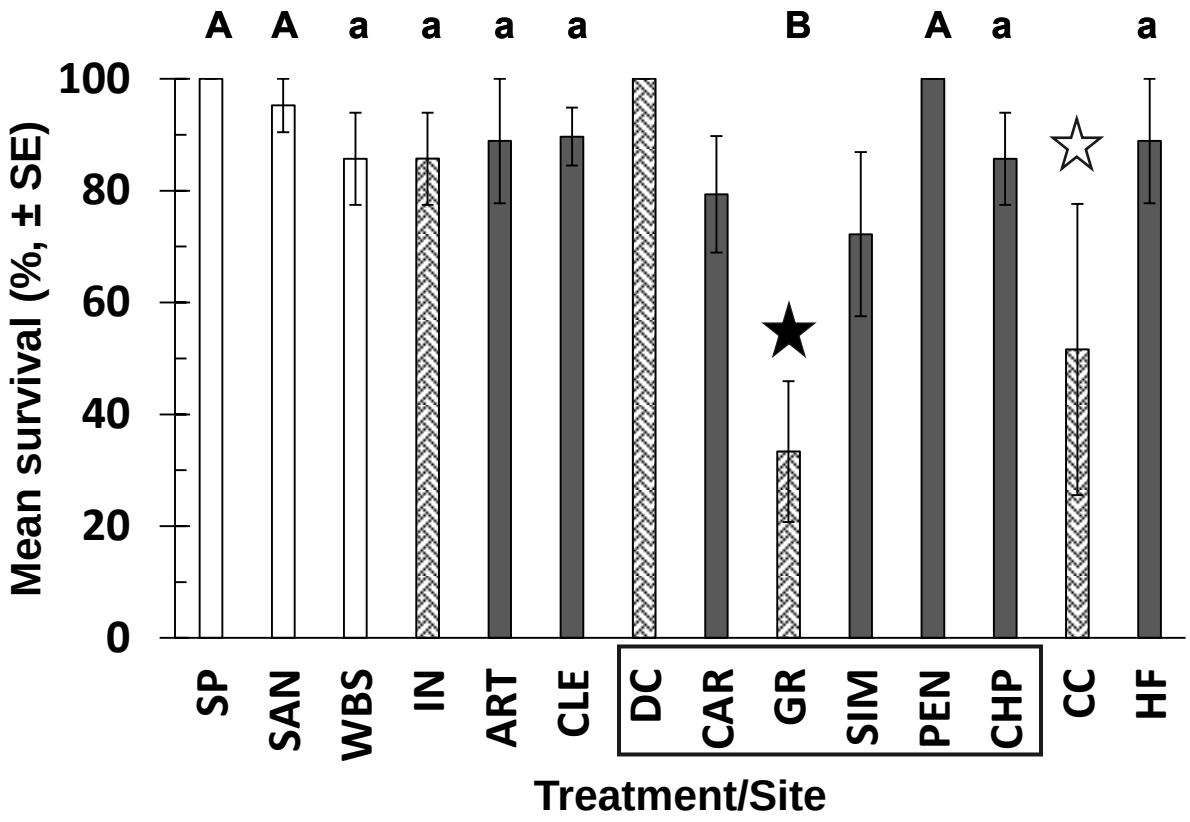

A A a a a a B A a a
100
80
60
40
20
0
Mean survival (%, ± SE)
SP
SAN
WBS
IN
ART
CLE
DC
CAR
GR
SIM
PEN
CHP
CC
HF
Treatment/Site

Figure 3.

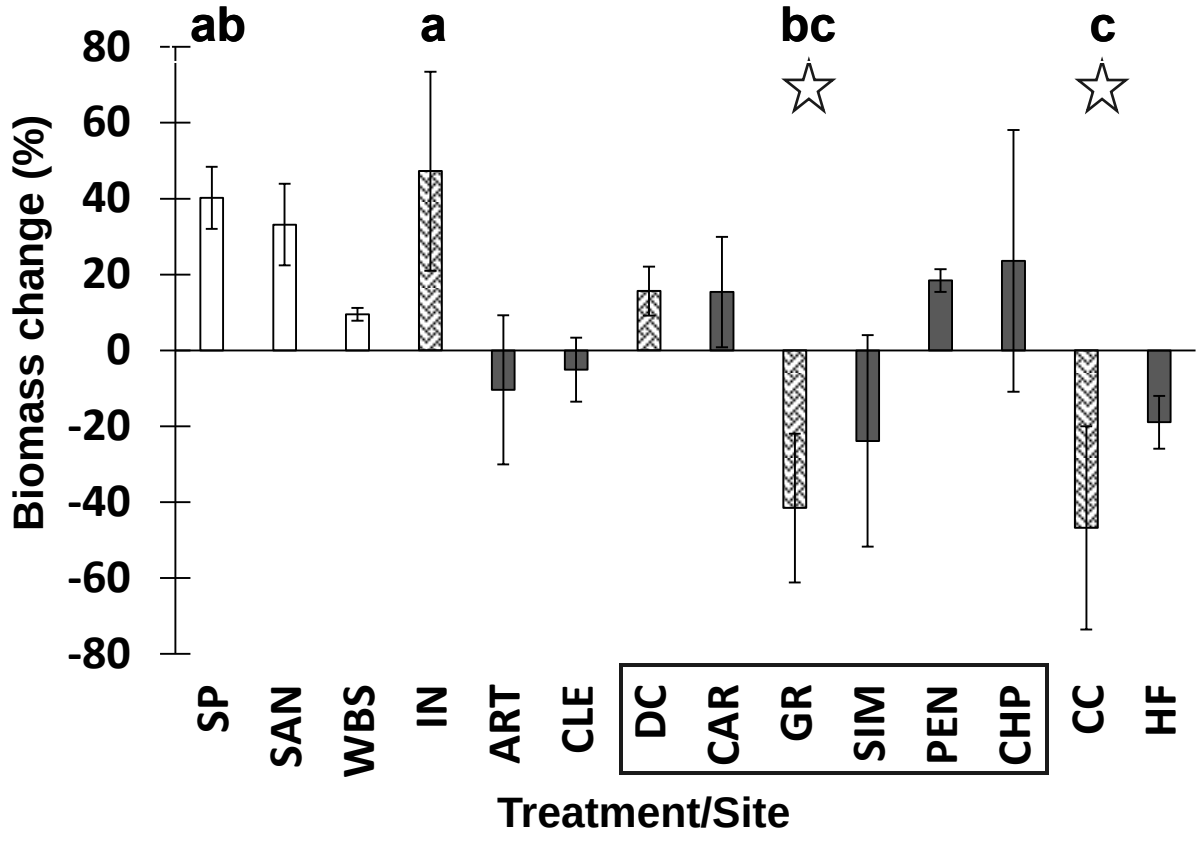

ab
a
bc
c
80
60
40
20
0
-20
-40
-60
-80
Biomass change (%)
SP
SAN
WBS
IN
ART
CLE
DC
CAR
GR
SIM
PEN
CHP
CC
HF
Treatment/Site

Figure 4.

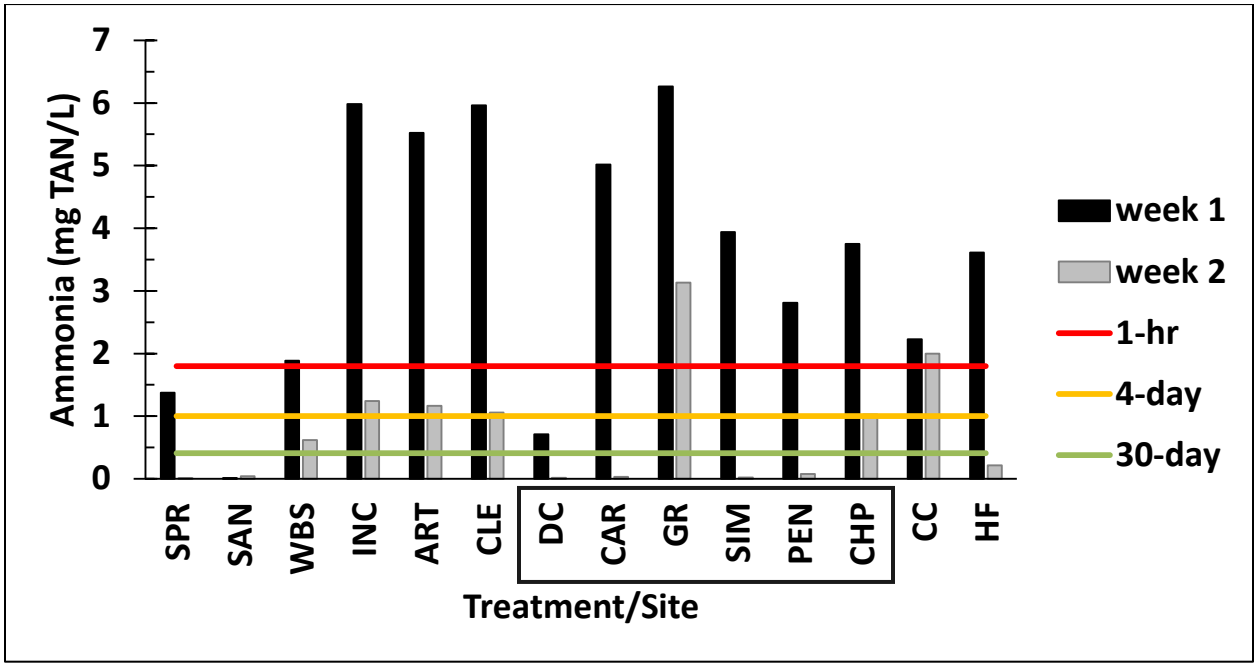

Ammonia (mg TAN/L)
0
1
2
3
4
5
6
7
SPR
SAN
WBS
INC
ART
CLE
DC
CAR
GR
SIM
PEN
CHP
CC
HF
Treatment/Site
week 1
week 2
1-hr
4-day
30-day

Figure 5.

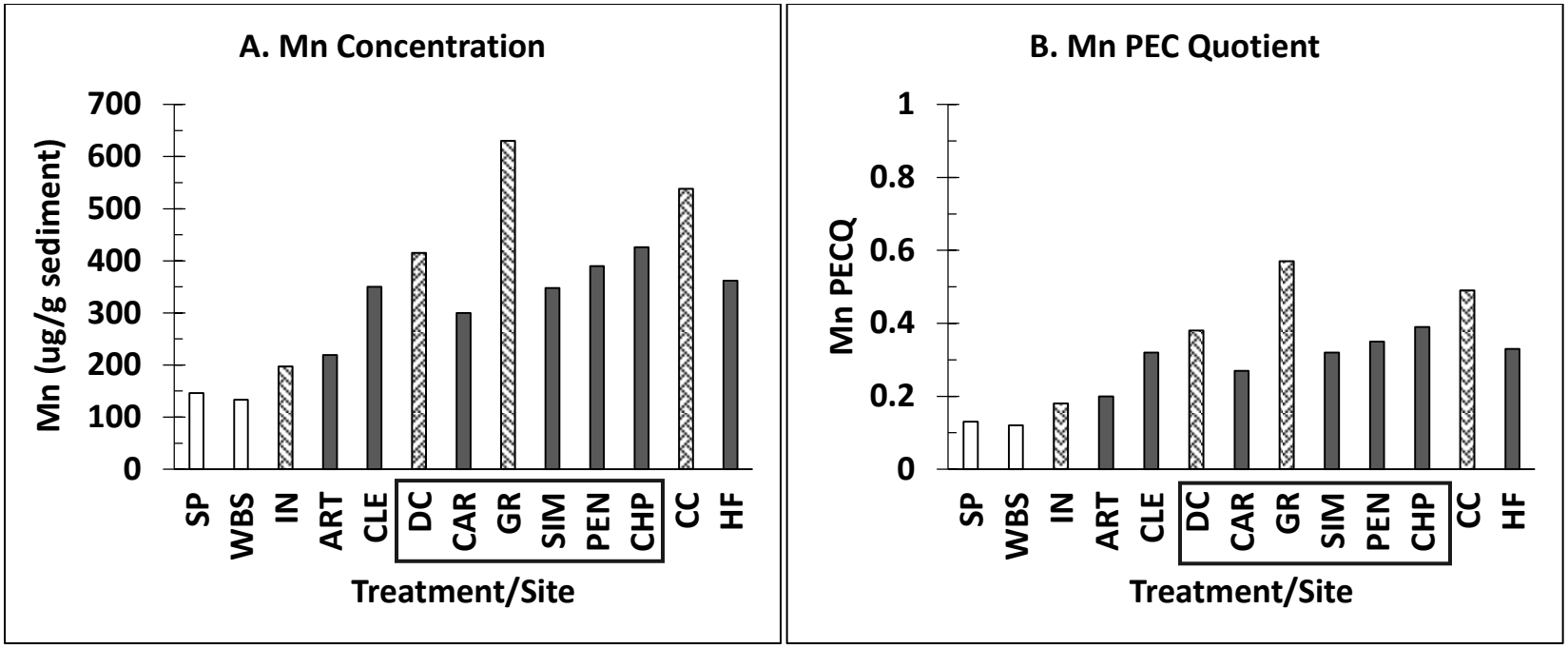
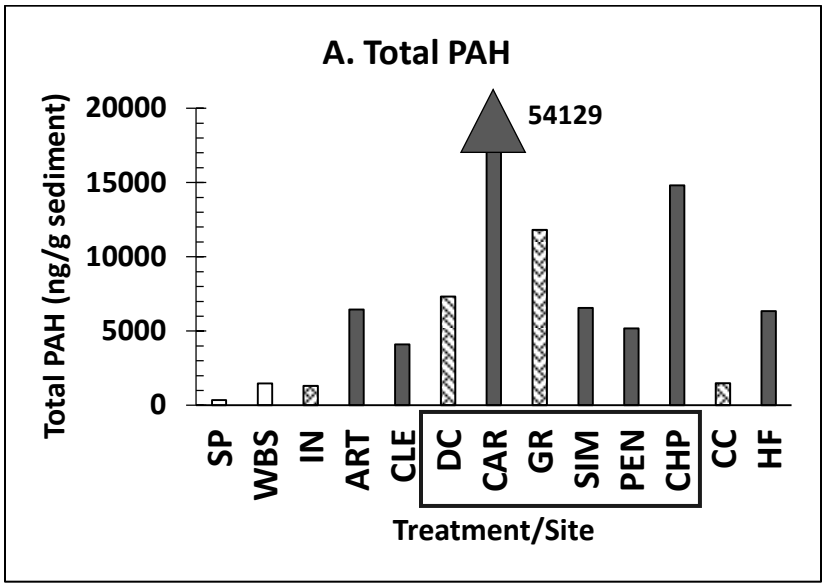

A. Mn Concentration
Mn (ug/g sediment)
700
600
500
400
300
200
100
0
SP
WBS
IN
ART
CLE
DC
CAR
GR
SIM
PEN
CHP
CC
HF
Treatment/Site
B. Mn PEC Quotient
Mn PECQ
1
0.8
0.6
0.4
0.2
0
SP
WBS
IN
ART
CLE
DC
CAR
GR
SIM
PEN
CHP
CC
HF
Treatment/Site

Figure 6.


A. Total PAH
Total PAH (ng/g sediment)
20000
15000
10000
5000
0
54129
SP
WBS
IN
ART
CLE
DC
CAR
GR
SIM
PEN
CHP
CC
HF
Treatment/Site

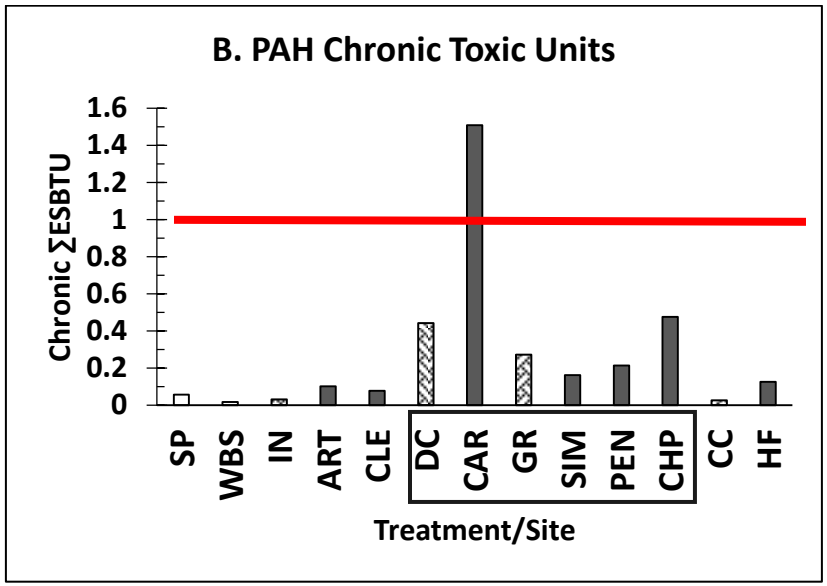

B. PAH Chronic Toxic Units
Chronic ∑ESBTU
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
SP
WBS
IN
ART
CLE
DC
CAR
GR
SIM
PEN
CHP
CC
HF
Treatment/Site

Figure 7.

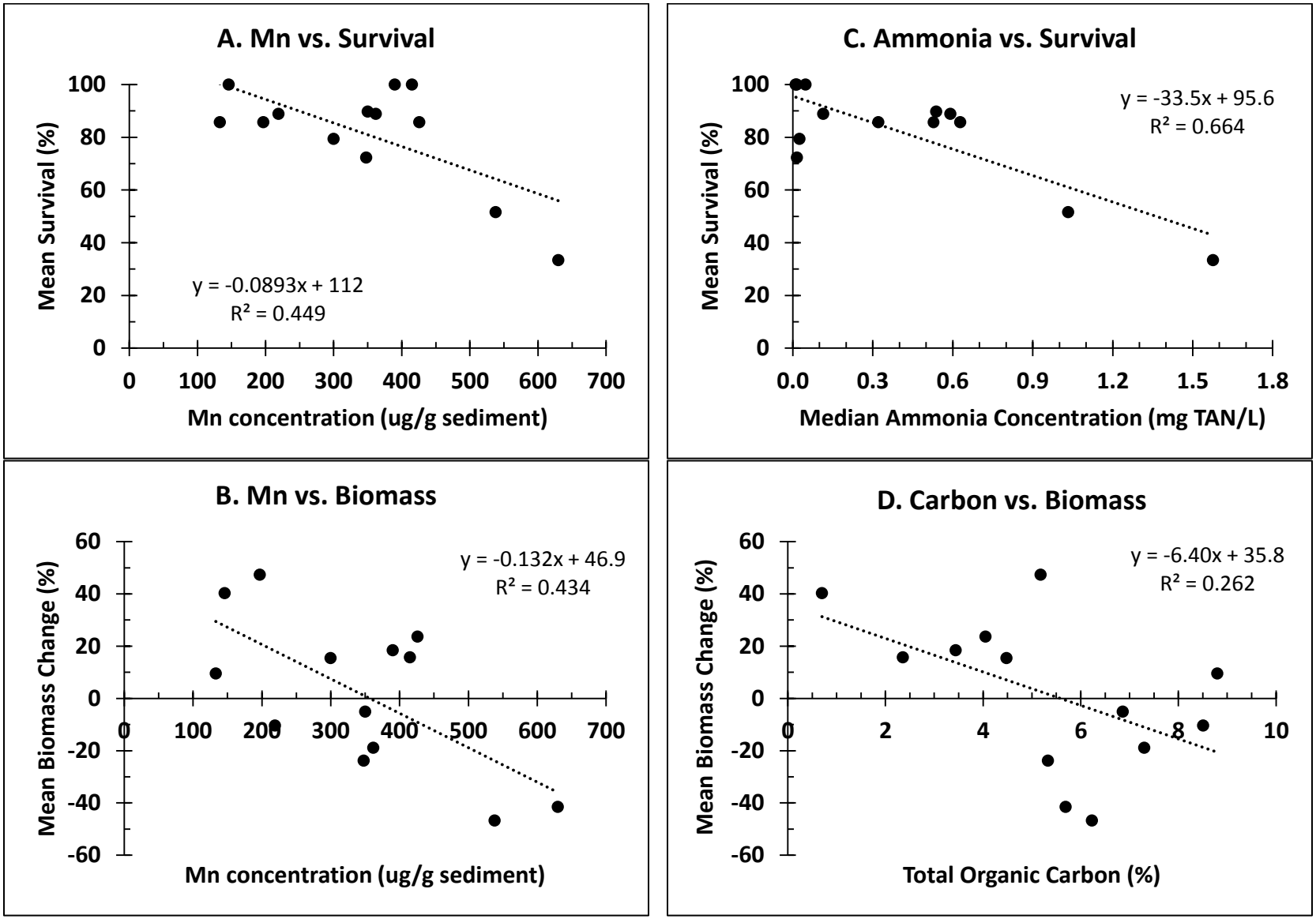
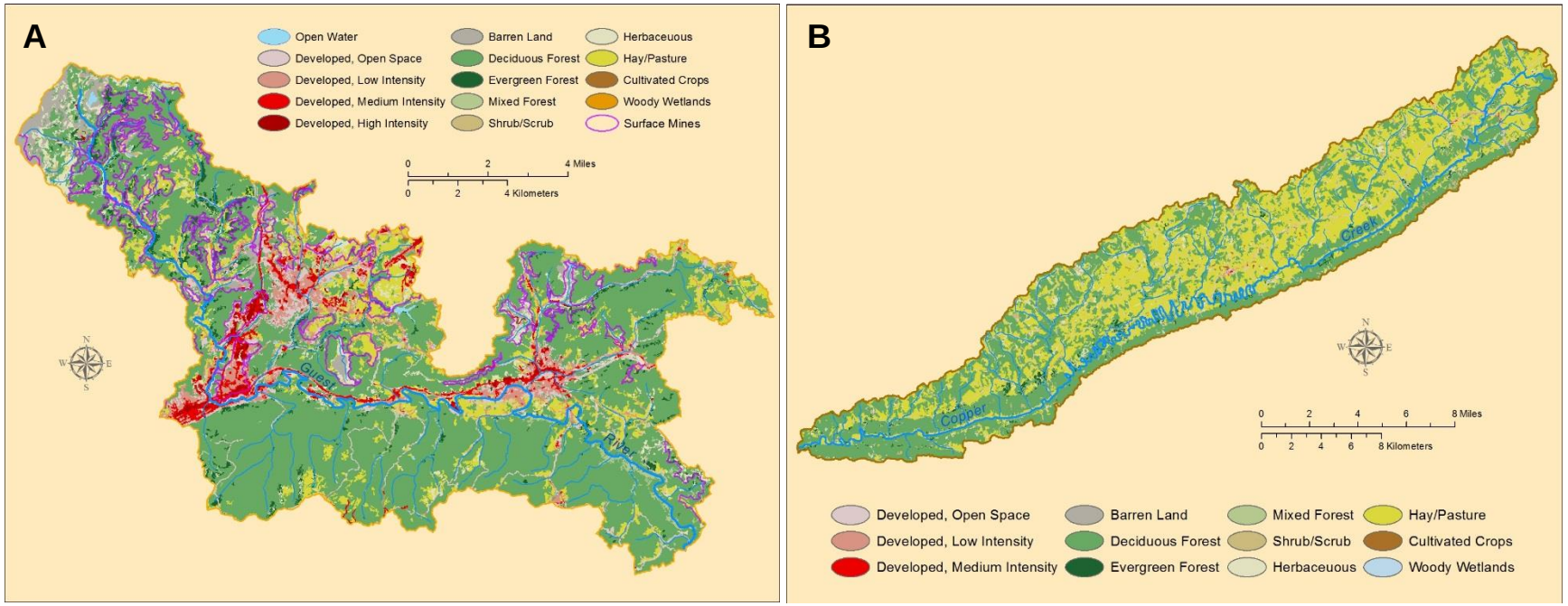

A. Mn vs. Survival
Mean Survival (%)
Mn concentration (ug/g sediment)
y = -0.0893x + 112
R² = 0.449
C. Ammonia vs. Survival
Mean Survival (%)
Median Ammonia Concentration (mg TAN/L)
y = -33.5x + 95.6
R² = 0.664
B. Mn vs. Biomass
Mean Biomass Change (%)
Mn concentration (ug/g sediment)
y = -0.132x + 46.9
R² = 0.434
D. Carbon vs. Biomass
Mean Biomass Change (%)
Total Organic Carbon (%)
y = -6.40x + 35.8
R² = 0.262

Figure 8.


A
Open Water
Developed, Open Space
Developed, Low Intensity
Developed, Medium Intensity
Developed, High Intensity
Barren Land
Deciduous Forest
Evergreen Forest
Mixed Forest
Shrub/Scrub
Herbaceuous
Hay/Pasture
Cultivated Crops
Woody Wetlands
Surface Mines
0 2 4 Miles
0 2 4 Kilometers
Guest
River
B
Copper
Creek
0 2 4 6 8 Miles
0 2 4 6 8 Kilometers
Developed, Open Space
Developed, Low Intensity
Developed, Medium Intensity
Barren Land
Deciduous Forest
Evergreen Forest
Mixed Forest
Shrub/Scrub
Herbaceuous
Hay/Pasture
Cultivated Crops
Woody Wetlands

Tables

Table 1. Probable effect concentration quotients (PECQs) of individual metals in each treatment for which Probable Effect Concentrations (PECs) are available (Persaud et al. 1993; MacDonald et al. 2000), and the mean metal PECQ for each sediment treatment. Metal concentrations (µg/g sediment) and PECs (mg/kg dry weight sediment) are in equivalent units; PECQ = sediment concentration/PEC. Treatment (site) names and abbreviations are as follows: Spring River, SP; Sand, SAN; West Bearskin, WBS; Indian Creek, IN; Artrip, ART; Cleveland, CLE; Dumps Creek, DC; Carterton, CAR; Guest River, GR; Simones, SIM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Clinch treatments shaded are those from within the mussel zone of decline.

Metal	PEC	Individual Metal PECQs for each Sediment Treatment												
		SP	WBS	IN	ART	CLE	DC	CAR	GR	SIM	PEN	CHP	CC	HF
As	33	.04	.07	.11	.06	.06	.11	.06	.06	.06	.06	.07	.10	.08
Cr	111	.07	.11	.07	.06	.07	.07	.07	.04	.06	.07	.08	.11	.09
Cu	149	.03	.15	.10	.09	.08	.16	.08	.09	.10	.08	.09	.08	.08
Fe	40000	.10	.30	.19	.17	.20	.26	.21	.16	.20	.19	.22	.23	.21
Mn	1100	.13	.12	.18	.20	.32	.38	.27	.57	.32	.35	.39	.49	.33
Ni	48.6	.10	.26	.11	.10	.12	.16	.12	.17	.13	.13	.15	.13	.12
Pb	128	.06	.04	.05	.06	.07	.07	.07	.06	.07	.06	.07	.08	.06
Zn	459	.12	.08	.06	.07	.07	.13	.07	.09	.07	.06	.07	.06	.06
Mean Metal PECQ		.08	.14	.11	.10	.12	.17	.12	.15	.13	.13	.14	.16	.13

Table 2. Pearson product-moment and Spearman rank-order correlation coefficients and p-values ($p < 0.05$ are bold) between mussel survival and biomass and significantly correlated sediment treatment constituent concentrations from the 28-day mussel toxicity test.

Parameter	**Survival**			
	Pearson	p-value	Spearman	p-value
Mn	-0.67	**0.01**	-0.27	0.38
Median ammonia	-0.81	**< 0.01**	-0.52	0.07
	Biomass			
	Pearson	p-value	Spearman	p-value
Mn	-0.66	**0.01**	-0.42	0.16
Carbon (%)	-0.51	0.07	-0.63	**0.02**

Table 3. Ranking of candidate linear regression models explaining trends in mussel survival and biomass by Akaike's Information Criterion, corrected for small samples (AIC_C), AIC_C differences (Δ_i), and model weight (w_i), followed by the most parsimonious models with parameter estimates and p-values for partial slopes. Ammonia = median total ammonia nitrogen concentration, Mn = manganese concentration, Carbon = sediment total organic percent, and PAH Chronic HQ = chronic hazard quotient for Total PAH.

	Candidate Model Selection	**Model Ranking** AIC_C	Δ_i	w_i
	Survival			
1	Ammonia	276.3102	0	0.42
2	Ammonia + Mn	276.3875	0.08	0.41
3	Ammonia + Mn + PAH Chronic HQ	278.1071	1.80	0.17
	Biomass			
1	Mn + Carbon	314.0853	0	0.69
2	Mn + Carbon + PAH Chronic HQ	316.4301	2.34	0.21
3	Mn + Carbon + PAH Chronic HQ + Ammonia	318.7827	4.70	0.07
4	Mn	320.6459	6.56	0.03
	Most Parsimonious Models	**Partial slope p-values** **Ammonia**	**Mn**	**Carbon**
	Survival			
	95.64 – 33.47(Ammonia)	< 0.01		
	106.62 – 26.33 (Ammonia) – 0.04(Mn)	< 0.01	0.14	
	Biomass			
	85.65 – 0.14(Mn) – 6.90(Carbon)		< 0.01	< 0.01

Supplemental tables.

Table S1. Particle size distribution and organic carbon content of sediments collected from the Clinch River and controls. Clinch River sediments are listed in upstream to downstream order.

Treatment/Site	Sand (%)	Silt (%)	Clay (%)	Carbon (%)
Spring River – Control	75.9	13.1	11.1	0.70
Sand – Control	99.7	--	--	0.09
West Bearskin – Control	54.0	39.6	6.4	8.80
Indian Creek	92.0	3.6	4.4	5.18
Artrip	71.6	20.0	8.4	8.51
Cleveland	42.5	44.5	13.0	6.86
Dumps Creek	86.3	8.7	5.0	2.36
Carterton	59.6	30.1	10.3	4.48
Guest River	80.8	14.0	5.3	5.69
Simones	68.3	24.6	7.1	5.33
Pendleton	77.4	16.5	6.1	3.44
Clinchport	60.0	29.8	10.2	4.05
Copper Creek	54.3	37.2	8.5	6.23
Horton Ford	81.4	12.4	6.3	7.30
Summary Statistics for Clinch River Basin Sediments				
Mean	70.4	21.9	7.7	5.40
Standard Deviation	15.0	12.6	2.7	1.79
Range	42.5 – 92.0	3.6 – 44.5	4.4 – 13.0	2.36 – 8.51

Table S2. Weekly ammonia concentrations (mg/L NH_3-N) and pH measured in the overlying water of each treatment during a 28-d exposure of juvenile freshwater mussels to recently-deposited sediments collected from the Clinch River Basin. The pH of the laboratory reconstituted hard water (ASTM 2007) is also reported. Clinch River treatments are listed from upstream to downstream order.

	Ammonia (mg/L NH_3-N)				
Treatment/ Site	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Median**
Spring River – Control	1.373	.013	.009	.008	**.011**
Sand – Control	.009	.041	.043	.058	**.042**
West Bearskin – Control	1.886	.620	.023	.012	**.321**
Indian Creek	5.985	1.240	.016	.015	**.628**
Artrip	5.523	1.163	.019	.012	**.591**
Cleveland	5.963	1.060	.016	.014	**.538**
Dumps Creek	.709	.018	.011	.007	**.015**
Carterton	5.018	.030	.015	.021	**.025**
Guest River	6.262	3.132	.021	.013	**1.576**
Simones	3.940	.023	.007	.008	**.015**
Pendleton	2.810	.079	.016	.010	**.048**
Clinchport	3.751	1.026	.030	.012	**.528**
Copper Creek	2.227	2.000	.066	.016	**1.033**
Horton Ford	3.610	.216	.012	.013	**.114**
	pH				
Treatment/ Site	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Mean**
Lab ASTM hard water	8.18	8.45	8.48	8.38	**8.37**
Spring River – Control	8.10	8.47	8.51	8.56	**8.41**
Sand – Control	8.27	8.37	8.40	8.57	**8.40**
West Bearskin – Control	7.08	7.84	7.72	8.23	**7.72**
Indian Creek	7.90	8.47	8.94	8.55	**8.47**
Artrip	7.63	8.63	9.07	8.65	**8.50**
Cleveland	7.83	8.93	8.96	8.58	**8.60**
Dumps Creek	8.44	8.61	8.42	8.63	**8.53**
Carterton	8.57	9.18	8.62	8.37	**8.69**
Guest River	7.82	7.96	8.84	9.03	**8.41**
Simones	8.12	9.10	8.75	8.35	**8.58**
Pendleton	7.92	8.71	8.89	8.56	**8.52**
Clinchport	7.80	8.39	9.03	8.70	**8.48**
Copper Creek	7.80	8.14	8.98	8.62	**8.39**
Horton Ford	8.00	8.56	8.78	8.54	**8.47**

Table S3. Metal concentrations (reported to at least three significant figures) measured in each of the sediment samples after collection and processing. Clinch River treatments are listed from upstream to downstream order after the three controls (SP, WBS, and SAN), and sediments highlighted in grey were collected at sites within the mussel zone of decline. Detection limits (DL) and Probable Effect Concentrations (PEC; Persaud et al. 1993, MacDonald et al. 2000) are provided, along with summary statistics, including the mean PEC quotient (PECQ) for each metal among the 11 Clinch River treatments. Dashes indicate a measurement was below DL.

			Clinch Summaries															
Metal	**DL**	**PEC**	**SP**	**WBS**	**SAN**	**IN**	**ART**	**CLE**	**DC**	**CAR**	**GR**	**SIM**	**PEN**	**CHP**	**CC**	**HF**	**Mean**	**Mean PECQ**	**Range**
Al	**0.5**		2143	2973	191	1929	2758	3710	2824	3514	2104	3070	2702	3540	3806	2660	2965		1929 - 3806
As	**0.5**	**33**	1.19	2.16	--	3.66	1.83	1.92	3.64	2.06	1.82	2.03	2.12	2.23	3.21	2.60	2.47	0.075	1.82 - 3.66
Ba	**0.5**		33.5	16.5	.723	31.5	52.3	58.4	44.8	57.6	34.9	42.0	38.0	44.2	43.9	35.6	43.9		31.5 - 58.4
Be	**0.25**		--	--	--	0.367	0.359	0.393	0.416	0.407	0.356	0.396	0.405	0.448	0.466	0.390	0.400		0.356 - 0.466
Cd	**0.25**	**4.98**	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Co	**0.5**		3.24	3.58	--	3.27	3.19	4.02	5.26	3.91	5.42	4.36	4.25	4.94	6.11	4.54	4.48		3.19 - 6.11
Cr	**0.5**	**111**	8.01	12.2	--	8.15	6.59	7.47	7.30	7.53	4.62	6.91	7.28	8.42	12.2	10.0	7.86	0.071	4.62 - 12.2
Cu	**0.5**	**149**	4.67	22.3	--	14.7	14.1	11.5	24.3	12.5	13.1	14.4	11.5	13.4	12.0	12.0	14.0	0.094	11.5 - 24.3
Fe	**0.5**	**40000**	3827	12004	220	7779	6825	7866	10212	8293	6426	7841	7771	8629	9215	8436	8118	0.203	6426 - 10212
Hg	**0.05**	**1.06**	--	--	--	--	--	0.051	--	--	--	--	--	--	--	--	--	--	--
K	**0.5**		191	343	--	317	412	476	488	431	265	462	453	589	541	465	445		265 - 589
Mg	**0.5**		211	1341	13.7	506	1921	1829	1024	1918	624	1467	959	1257	1589	1187	1298		506 - 1921
Mn	**0.5**	**1100**	146	133	--	197	219	350	415	300	630	348	390	426	538	362	379	0.345	197 - 630
Mo	**0.25**		--	1.91	--	--	--	--	--	0.786	--	--	--	--	--	--	--	--	--
Ni	**0.5**	**48.6**	4.64	12.6	--	5.45	4.71	5.92	7.68	6.04	8.37	6.49	6.28	7.32	6.45	5.83	6.41	0.132	4.71 - 8.37
Pb	**0.25**	**128**	7.983	4.851	--	5.771	7.623	9.294	9.007	8.365	7.388	8.558	7.240	8.973	10.790	7.771	8.25	0.064	5.77 - 10.8
Sb	**0.25**		--	0.760	--	0.501	0.490	0.450	0.632	0.479	0.408	0.493	0.469	0.355	0.601	0.581	0.496		0.355 - 0.632
Se	**0.5**		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Si	**0.5**		302	281	17.4	134	227	297	203	237	160	229	232	305	278	197	227		134 - 305
Sr	**0.5**		2.97	3.90	--	7.91	19.4	18.3	22.5	17.9	11.1	16.5	11.9	13.4	8.03	13.0	14.5		7.91 - 22.5
V	**0.5**		7.34	18.0	.583	6.66	11.0	12.4	8.92	12.5	6.41	10.6	9.20	11.3	14.6	10.7	10.4		6.41 - 14.6
Zn	**0.5**	**459**	56.7	36.6	1.20	29.6	30.2	34.1	57.8	33.1	40.6	34.4	29.0	34.1	27.0	25.3	34.1	0.074	25.3 - 57.8
Total metals			6952	17207		10979	12514	14693	15358		10343	14855	12636	14895	16112	13435			

Table S4. Polycyclic aromatic hydrocarbon (PAH) concentrations measured in each of the sediment samples after collection and processing. Clinch River treatments are listed from upstream to downstream order after the controls (SP and WBS), and sediments highlighted in grey were collected at sites within the mussel zone of decline. The sand control was not analyzed for organic contaminants because it had essentially no organic content. The sum of all 42 PAH (i.e., Total PAH) and sum of the 34 PAH used in calculating equilibrium-partitioning sediment benchmark toxic units (∑ESBTUs) are provided, along with acute and chronic toxic units, and percent petrogenic and pyrogenic for each sediment.

PAH	Individual PAH concentrations in Sediments (ng/g dry weight sediment)												
	SP	WBS	IN	ART	CLE	DC	CAR	GR	SIM	PEN	CHP	CC	HF
Naphthalene	0.0	18.7	9.1	99.3	50.7	126.7	348.9	132.3	100.3	102.8	133.6	8.8	144.7
Acenaphthylene	0.3	1.0	1.4	5.1	6.5	2.5	366.9	35.0	13.3	7.9	91.1	16.9	2.8
Acenaphthene	0.0	0.0	0.0	8.8	18.0	27.2	93.8	13.7	11.2	9.1	22.8	0.0	9.1
C1 - Naphthalenes	3.5	22.0	25.6	346.1	184.8	564.6	503.0	571.3	401.3	413.6	476.2	16.5	579.5
C2 - Naphthalenes	14.5	0.0	87.3	686.1	423.6	883.1	938.7	1071.3	692.4	653.6	847.2	0.0	909.7
C3 - Naphthalenes	12.3	0.0	67.6	503.8	305.9	615.0	703.5	971.2	504.7	502.7	718.8	0.0	686.1
C4 - Naphthalenes	13.4	0.0	38.4	239.1	142.4	228.2	279.8	507.7	229.5	242.7	364.8	0.0	318.0
Fluorene	1.1	16.2	5.8	24.4	29.6	52.3	250.8	37.4	20.9	21.7	51.1	2.0	23.1
C1 - Fluorenes	4.0	34.3	20.3	62.6	52.3	111.8	190.8	112.5	63.9	64.8	124.6	0.0	71.1
C2 - Fluorenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C3 - Fluorenes	0.0	0.0	61.1	155.4	111.4	182.6	0.0	299.1	151.6	139.8	453.4	0.0	146.8
Dibenzothiophene	1.5	1.6	5.8	17.8	12.7	30.6	403.4	30.0	16.6	14.8	65.9	0.0	18.4
C1 - Dibenzothiophene	6.6	0.0	20.5	46.2	30.3	54.5	251.2	85.5	44.7	40.6	97.0	0.0	46.6
C2 - Dibenzothiophene	9.2	0.0	19.3	41.0	31.1	49.8	172.9	85.1	42.4	35.3	90.3	10.1	40.0
C3 - Dibenzothiophene	5.7	0.0	12.3	0.0	17.6	25.6	71.9	51.9	25.6	19.9	44.0	10.4	21.3
Phenanthrene	12.8	67.8	67.6	322.2	177.1	496.3	8290.2	414.3	253.1	235.2	1277.9	13.0	319.9
Anthracene	1.1	8.3	6.1	49.2	22.5	39.5	401.2	64.6	38.0	26.9	101.3	27.8	27.5
C1 - Phenanthrenes/Anthracenes	21.0	0.0	131.5	507.0	298.7	669.1	3298.2	937.6	497.9	479.4	1277.2	30.0	614.5
C2 - Phenanthrenes/Anthracenes	32.9	0.0	135.7	494.8	300.2	523.9	1561.5	958.1	473.8	429.4	976.8	78.4	532.6
C3 - Phenanthrenes/Anthracenes	20.4	0.0	72.7	219.9	132.9	216.5	513.5	497.1	217.1	191.2	419.3	49.5	241.4
C4 - Phenanthrenes/Anthracenes	12.8	0.0	29.7	111.3	66.6	87.3	195.5	223.4	117.7	100.5	173.9	29.4	119.5
Fluoranthene	8.9	41.3	39.1	284.3	169.4	296.5	7531.3	334.5	179.2	83.8	1176.7	100.5	72.0
Pyrene	6.6	22.1	31.9	200.1	137.2	227.1	5606.9	348.6	177.5	77.5	914.7	94.6	63.6
C1 - Fluoranthenes/Pyrene	7.9	26.5	36.0	195.1	108.5	189.8	1608.2	357.3	202.0	126.5	419.3	93.9	141.3
C2 - Fluroanthrene/Pyrene	14.8	0.0	54.3	213.1	141.5	232.2	2023.6	448.9	240.7	182.2	587.2	73.1	212.3
C3 - Fluoranthrene/Pyrene	8.9	0.0	50.4	180.0	86.2	178.3	682.7	337.3	180.3	145.1	219.2	34.0	169.9
Retene	3.6	97.6	2.9	16.7	11.0	8.1	35.4	54.1	20.2	17.9	30.1	5.5	39.1
Benz[a]anthracene	2.7	9.9	15.3	126.6	61.9	91.0	1221.3	246.5	91.3	49.9	308.7	69.3	40.8

Table S4 continued.

PAH	Individual PAH concentrations in Sediments (ng/g dry weight sediment)												
	SP	WBS	IN	ART	CLE	DC	CAR	GR	SIM	PEN	CHP	CC	HF
Chrysene	6.6	21.8	43.3	195.5	132.2	170.1	3003.7	348.9	148.7	108.8	566.4	155.0	97.7
C1 - Chrysenes	8.7	0.0	32.2	119.3	71.1	118.9	809.5	273.9	97.7	92.2	264.3	49.4	103.6
C2 - Chrysenes	0.0	0.0	25.7	91.4	56.9	94.4	298.9	228.1	83.0	77.2	137.4	19.6	90.6
C3 - Chrysenes	0.0	0.0	15.2	53.3	36.6	53.7	147.4	127.2	52.4	46.5	72.4	0.0	55.2
C4 - Chrysenes	0.0	0.0	11.0	51.4	39.4	48.9	333.4	122.9	43.3	51.9	76.6	0.0	49.1
Benzo[b]fluoranthene	5.5	42.5	27.7	137.8	120.2	96.1	2857.8	320.3	162.5	75.2	419.1	108.5	54.5
Benzo[k]fluoranthene	3.4	16.2	11.8	96.6	72.8	73.2	1616.0	221.9	94.0	38.4	373.1	102.9	27.1
Benzo[e]pyrene	4.9	0.0	19.7	116.9	84.3	95.5	1582.4	232.5	127.2	66.9	310.1	63.1	56.9
Benzo[a]pyrene	5.1	0.0	19.0	107.5	86.3	88.2	1549.0	220.6	127.3	66.1	308.7	63.1	55.6
Perylene	91.3	874.1	10.3	57.2	62.4	53.2	383.5	69.1	62.6	31.6	112.6	62.2	31.0
Indeno[1,2,3-cd]pyrene	6.5	47.3	13.4	117.0	84.9	69.0	1918.7	166.2	194.0	37.9	313.4	42.7	27.8
Dibenz[a,h,]anthracene	0.0	0.0	3.6	28.4	18.2	18.2	274.2	47.6	46.4	11.9	69.8	8.8	10.7
Benzo[g,h,i]perylene	5.8	43.7	12.2	98.0	74.9	81.5	1460.9	158.9	192.4	46.6	256.3	28.0	39.6
Coronene	0.0	71.7	5.4	23.7	20.8	41.5	348.9	36.8	122.0	22.8	58.7	9.7	23.7
Totals													
Sum of 42 PAH (Total PAH)	364	1484	1298	6450	4092	7312	54129	11801	6561	5189	14802	1477	6335
Sum of 34 PAH	314	1314	1127	5912	3740	6692	50139	10672	5868	4710	13610	1334	5763
∑ESBTUs													
Acute TU	0.014	0.004	0.007	0.025	0.019	0.106	0.368	0.066	0.039	0.051	0.116	0.006	0.031
Chronic TU	0.057	0.018	0.031	0.103	0.079	0.442	1.509	0.272	0.162	0.214	0.477	0.026	0.127
Contribution by PAH Type													
Petrogenic (%)	73.9	23.2	75.3	69.9	68.0	73.6	29.0	72.5	69.5	81.2	55.2	35.8	85.2
Pyrogenic (%)	26.1	76.8	24.7	30.1	32.0	26.4	71.0	27.5	30.5	18.8	44.8	64.2	14.8