

Environmental Toxicology

ASSESSING TOXICITY OF CONTAMINANTS IN RIVERINE SUSPENDED SEDIMENTS TO FRESHWATER MUSSELS

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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. The authors 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 6 sites within an 88-km reach deemed a "mussel zone of decline." Mussels were exposed to the riverine sediments and to 3 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 2 of the tributaries were the most toxic. Inorganic analyses of sediments indicated the presence of metals and polycyclic aromatic hydrocarbons at all sites. Manganese was negatively correlated with mussel survival and biomass, as was ammonia with survival and total organic carbon with biomass. Current land uses in the watershed indicate that fossil fuel mining and agriculture may be associated with elevated manganese and ammonia, respectively. The authors found that sediments collected with sediment traps over relatively short deployment durations can help elucidate recent contaminant influx and its potential for inducing toxicity in benthic organisms. *Environ Toxicol Chem* 2016;9999:1–13. © 2016 SETAC

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INTRODUCTION

The southeastern United States is the richest region of global diversity for freshwater fish, crayfish, and mussel species [1] and is, therefore, a region of high conservation priority. However, this high regional biodiversity intersects with intense pressures of fossil fuel 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 fishes in North America [2] lies the Clinch River. More than 135 freshwater species in the province have been identified as priorities for conservation, most of which are fishes and mollusks [3]. The Clinch River alone has supported approximately half of the region's mussel and fish species [3,4]; many of those fishes likely serve as hosts to the parasitic larval stage of freshwater mussels [5].

Currently, approximately 18% of the known mussel fauna are believed to be extirpated from the river [6]. 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 [7], including 20 endangered mussel species [6]. Much of this decline has been documented over the last 35 yr [6], 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 as much as 96% [6] (Figure 1).

Investigations into possible reasons for such declines were recently published in a series of journal articles and summarized by Zipper et al. [4]. Physical habitat was ruled out as a limiting factor, and pollution was the overarching theme. Analyses of data sets for water and sediment quality spanning from the 1960s to 2013 indicated that excessive ammonia from poor wastewater treatment was a major issue in the 1970s and 1980s, though surface water concentrations have improved considerably, especially in the most recent decade [8]. Metal concentrations, including those released in 2 spills (fly ash in 1967 and sulfuric acid in 1970) from a coal-fired power plant on the banks of the Clinch River near Carbo, were highest in the 1980s and 1990s and have declined over time [6,8]; but some metals may still be a cause of concern (e.g., iron [Fe] and manganese [Mn]) [9]. Unlike ammonia and metals, dissolved solids, major ions, and specific conductance have trended upward over time and were negatively associated with mussel assemblage quality [4,8,9]. 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 [4,9].

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Figure 1. Map of study area and sediment collection sites in the Clinch River watershed. There were 8 mainstem (circles) and 4 tributary (triangles) sites. The zone of documented mussel decline is denoted by the red box and includes Dumps Creek, Carterton, Guest River, Semones, Pendleton, and Clinchport.

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 because of poorer benthic macroinvertebrate communities found in streams affected by mining activities [10]. 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 [11], which may also impact aquatic fauna [12]. 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 [13]. Given the myriad historic and ongoing pollutant issues in the Clinch River, identifying and mitigating the causative agent(s) for declining mussel populations, along with other fauna, has proven difficult; and there is concern that chronic toxicity arising from nonpoint sources and potentially from toxic mixtures is a likely contributor.

Several studies involving the Clinch River and its watershed have focused on surface water quality, bed sediments, physical habitat, and land use. Although some water quality parameters are related to runoff and sedimentation, no study has specifically addressed the toxic effects of contaminants in the suspended sediment load, an important ecological compartment in riverine systems. The objectives of the present study were to 1) determine whether fine particulate sediments in the water column (i.e., the suspended 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 suspended sediment load and quantify their concentrations, 3) identify the contaminants that may be related to any toxicity observed in mussels, and 4) consider how our results may inform improved conservation and management in the Clinch River watershed.

MATERIALS AND 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 [6]. Eight mainstem river and 4 tributary sites were distributed upstream of (n=3), within (n=6), and downstream of (n = 3) the zone of mussel decline (Figure 1). Indian Creek (a tributary), Artrip, and Cleveland (Virginia, USA) are located upstream; Dumps Creek (a tributary), Carterton, Guest River (a tributary), Semones, Pendleton, and Clinchport (Virginia, USA) are within the zone of decline; and Copper Creek (Virginia, USA, a tributary), Horton Ford, and Wallen Bend (Tennessee, USA) are downstream of the zone of decline. Copper Creek enters the Clinch River from the southeast and drains the valley and ridge physiographic landforms, while Indian and Dumps Creeks and the Guest River enter from the northwest, draining the coal-rich and gasrich Appalachian Plateau physiographic region. The Guest River currently does not support mussels [9], though as a major tributary it drains a substantial portion of the Clinch River watershed.

Sediment collection and processing

Sediment traps were constructed from polyvinyl chloride pipe (30.5 cm length \times 7.6 cm diameter), had a removable bottom, and were topped with a sheet of plastic 1-cm mesh to keep out large debris (both fastened on with stainless steel hose clamps). Two sediment traps were secured at each site within the openings of a 2-compartment cement block that was held in place by rebar. Traps were designed with an optimal aspect ratio for collecting sediments in lotic conditions [14] and deployed for approximately 5 mo (August 2013–January 2014), providing a time-integrated particulate sediment sample with sufficient volume for the experimental design and contaminant analyses. The volume of each trap was 1384 mL (i.e., sediment collection potential of $\geq 2 L$ per site), and most traps were full or nearly so at the time of collection. Sediment samples were placed in 950-mL amber glass jars certified to meet/exceed US Environmental Protection Agency (USEPA) standards for metal, pesticide, and semivolatile analyses (Thermo Scientific ICHEM series) and immediately transported on ice to the laboratory, where they were transferred to a refrigerator 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, and sampled for particle size, total organic carbon (TOC) content characterization, and contaminant analysis [15]. Afterward, they were returned to the refrigerator for a total holding time of 2 wk before test initiation to ensure the lack of indigenous organisms.

Experimental design and conditions

We evaluated the toxicity of the Clinch River suspended sediments to juvenile freshwater mussels in a 28-d staticrenewal exposure with 200 mL of test sediments and 800 mL reconstituted hard water [16] in each of 4 replicate 1-L beakers per treatment. Sediment and water were added to the test beakers and allowed to settle for 3 d to avoid exposing mussels to turbid conditions; 7 mussels were added to each of 3 replicates afterward (= test day 0). One replicate did not contain mussels, so we could evaluate potential contaminant loss via uptake by mussels versus leaching into the dissolved fraction in overlying water. Polyethylene and universal passive sampling devices [17] were randomly placed into the overlying water of 1 replicate in each treatment to measure the dissolved fraction of organic contaminants, enabling an understanding of chemical bioavailability from the test sediments.

Sediments from 11 of the 12 the Clinch River watershed sites (we were unable to recover the sediment traps from the Wallen Bend site) and 3 uncontaminated control sediments comprised the 14 test treatments. Two of the control sediments, Spring River (upper Spring River basin, MO, USA) and West Bearskin (from West Bearskin Lake, MN, USA) have been previously characterized and are routinely used in toxicity testing with benthic macroinvertebrates [18–20]. The third control sediment was a commercially available, contaminant-free filter sand (Southern Products and Silica), which has been used in previous toxicological studies with freshwater mussels [12].

The test was conducted in light-controlled and temperaturecontrolled environmental chambers (Precision Model 818; Thermo Fisher Scientific), held at 20 °C, on a 16:8-h light:dark cycle, and followed guidelines for water-only toxicity testing with early-life stage freshwater mussels [21] but modified as needed to accommodate sediment test conditions. Overlying water was renewed in each beaker 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 beaker sides with a peristaltic pump. Water quality was sampled weekly; mean conditions among all treatments were 164 mg calcium carbonate (CaCO₃)/L alkalinity, 178 mg CaCO₃/L hardness, 547 µS/cm conductivity, 8.43 pH, and 7.77 mg/L dissolved oxygen (n = 60). Individual treatment means ranged 84 mg to 209 mg CaCO₃/L alkalinity, 156 mg to 200 mg CaCO₃/L hardness, 520 µS/cm to 578 µS/cm conductivity, 7.72 to 8.69 pH, and 6.98 mg/L to 8.89 mg/L dissolved oxygen (n = 15). In addition, overlying water samples for total ammonia nitrogen analysis were collected weekly from all treatments to ensure that feeding and excretion did not cause adverse concentrations and to monitor any potentially toxic releases of ammonia from the test sediments. Ammonia samples were 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, NC, USA).

Though it was assumed that the volume of natural sediments in each beaker should provide ample nutrition over the duration of the test [22,23], 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; Reed Mariculture) concentrate diluted in deionized water was prepared, and approximately 5 mL was added to each replicate (administered concentrations of 25 000 cells/mL and 425 000 cells/mL solution, respectively) every 72 h after water chemistry samples were collected and at least 2 h before each water renewal. The sand treatment received 10 mL of food (administered concentrations of 50 000 cells/mL and 850 000 cells/mL solution, respectively) to compensate for a lack of organic material (i.e., natural food source) in the substratum [24,25].

Test organisms

The early life stages of freshwater mussels are important in toxicity testing because they are particularly susceptible to toxicants and other environmental stressors, in large part because of the unique parasitic (on a host fish) and multistage life cycle of these mussels. Juvenile sensitivity is often greater because they have less capacity to avoid exposure than adults [26]. Therefore, juvenile Cumberlandian combshell mussels (Epioblasma brevidens) were used in the present study to test the toxicity of the suspended sediment fraction collected from the Clinch River. 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 [27]. Juveniles used in the test were propagated via host-fish infection (Aquatic Wildlife Conservation Center, Virginia Department of Game & Inland Fisheries, Marion, VA, USA) from an adult female mussel collected from the Clinch River at Clinchport, Virginia, using standard propagation and culture methods [28]. Test mussels were shipped from the hatchery via overnight courier and, on arrival, slowly acclimated from culture water to test water and to 20 °C following ASTM International guidelines [21]. Following acclimation, mussels were held in an incubator and aerated until the experiment commenced. At the beginning of the exposure, the juveniles were 6 mo old with a mean initial shell length of $1.91 \text{ mm} (\pm 0.27 \text{ mm})$ standard deviation).

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 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%). The TOC content 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 silicon dioxide and contained <0.1% TOC (Supplemental Data, Table S1).

Contaminant analysis

Chemicals were measured in subsamples of each sediment collected before they were added to the test beakers, at the end of the exposure, and in the dissolved phase in the overlying water during the test with polyethylene and universal passive sampling devices 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, approximately one-half are listed as priority pollutants [29]. Quality assurance protocols were followed for all contaminant analyses, and quality control measures were within acceptable ranges (Supplemental Data).

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 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 5-min observation period were considered alive [21]. 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) for determination of biomass (total dry wt of surviving mussels in a replicate). Mussel biomass was normalized to a baseline sample of 35 mussels (5 replicates of 7 mussels) collected and weighed at the beginning of the experiment.

Differences in mean survival, percentage of length increase, and departure from baseline biomass among treatments were determined by analysis of variance (ANOVA; PROC GLM; SAS Ver 9.4; SAS Institute). Findings of significance ($\alpha = 0.05$) were followed by Dunnett's post hoc analyses to identify treatments that were different from the mean of the 3 control treatments (sand, Spring River, West Bearskin; $\alpha = 0.10$, exact *p* values reported). Prior to the Dunnett test, an ANOVA was conducted to confirm that mussel responses in the control treatments were not statistically different.

Characterizing sediment toxicity

Sediment quality guidelines provided probable effect concentrations (PEC) as an indicator of toxicity to freshwater organisms for 9 of the 22 metals analyzed (arsenic [As], cadmium [Cd], chromium [Cr], copper [Cu], Fe, Mn, nickel [Ni], lead [Pb], and zinc [Zn]) [30,31]. Individual PEC quotients 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 PEC quotient for each sample. An average PEC quotient of >1.0 is considered toxic to aquatic life; however, these sediment quality guidelines were derived from data sets that do not include toxicity to unionoid mussels. Recent research by others has found that sediments with values <0.4 can be toxic to mussels, indicating that current PECs may not reflect mussel toxicity [20]; and our results are considered in this context.

Toxicity of PAH concentrations was evaluated using the most up-to-date USEPA 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 [32]. Raw individual PAH concentrations in each sediment sample were normalized to the TOC fraction and then divided by the respective acute or chronic USEPA potency divisors (available for 34 PAHs) [32] to calculate the equilibriumpartitioning sediment benchmark toxic units. The individual PAH equilibrium-partitioning sediment benchmark toxic units were totaled; the resulting sum equilibrium-partitioning sediment benchmark toxic unit (hereafter referred to as "toxic unit") 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 to provide comparison with previous studies that have used that approach.

Measured total ammonia nitrogen concentrations were evaluated in the context of the recently updated ammonia water quality criteria for aquatic life [33]. 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 reduced the criteria values from previously published iterations. Our total ammonia nitrogen results are reported and discussed for comparison with the current ammonia criteria, which include acute and chronic values for concentrations allowed in surface waters. The acute criterion is defined as a 1-h average concentration at a given temperature and pH that should not be exceeded more than once every 3 yr [33]. The chronic criterion is defined as a 30-d rolling average that should not be exceeded more than once every 3 yr on average; further, the highest 4-d average within a 30-d period should not exceed 2.5 times the chronic criterion more than once in 3 yr [33]. For example, the acute and chronic criteria under conditions of 20 °C and 7.0 pH are 17 mg and 1.9 mg total ammonia nitrogen/L, respectively, whereas the 4-d limit is 4.8 mg total ammonia nitrogen/L (2.5 times the chronic criterion) under the same conditions.

To gain a better understanding of the variables potentially influencing the significant effects of sediment treatments on the measured endpoints, 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, Ver 9.4). Pearson product—moment correlations were used where relationships between a parameter and a response were linear, and Spearman rank-order correlation coefficients were used if a relationship was nonlinear or there were extreme values. Correlated variables were used to populate explanatory models for a given response, thus producing quantitative estimates of effects parameters (PROC GLMSELECT, SAS, Ver 9.4). The most plausible, parsimonious models explaining the affected mussel endpoints were selected from all possible Recently deposited sediment toxicity to freshwater mussels

models using Akaike's information criterion adjusted for low sample sizes (AIC_C) [34].

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 3 of the treatments, including 1 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 the Guest River (p = 0.005) and Copper Creek (p = 0.087) sediments had reduced survival (Figure 2).

Mean biomass of mussels in the baseline samples was $4.661 \text{ mg} (\pm 0.219 \text{ mg}, \text{ standard error})$. Biomass was significantly affected by sediment treatment (p=0.02); mean departures from the baseline at the end of the exposure ranged from -46.8% (Copper Creek) to +47.3% (Indian Creek). Mussels in all 3 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 6 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 Semones sediments were negative; but confidence limits crossed the line of no change (Figure 3). Mussels in the Copper Creek and Guest River sediments had reduced biomass when compared to control mussels (p = 0.064 and 0.098, respectively; Figure 3).

Percentage of length increase was not affected by sediment treatment (p = 0.37). Length increase overall was minimal (mean increase = 3.3%) and varied within and among treatments. The greatest increase was observed in the Spring River control sediment (6.6%), and the smallest percentage increase was in the sand control (1.3%). Mussel length measurements in



Figure 2. Mean survival (\pm standard error) in a 28-d test of freshwater mussels exposed to control (white bars) and Clinch River (gray bars = mainstem; hatched bars = tributary; arranged in order from upstream to downstream) sediments. Sites in the documented zone of mussel decline are denoted by the box. There was a significant effect of sediment treatment on survival (analysis of variance p < 0.01). Dunnett's post hoc test revealed lower survival compared to control in the Guest River and Copper Creek treatments (exact p values shown). ART = Artrip; CAR = Carterton; CC = Copper Creek; CHP = Clinchport; CLE = Cleveland; DC = Dumps Creek; GR = Guest River; HF = Horton Ford; IN = Indian Creek; PEN = Pendleton; SAN = sand; SEM = Semones; SP = Spring River; WBS = West Bearskin.



Figure 3. Mean change in biomass (\pm standard error) in a 28-d test of freshwater mussels exposed to control (white bars) and Clinch River (gray bars = mainstem; hatched bars = tributary; arranged in order from upstream to downstream) sediments compared to initial baseline biomass samples. Sites in the documented zone of mussel decline are denoted by the box. Treatments with bars above 0 gained biomass, and those below 0 lost biomass. There was a significant effect of sediment treatment on biomass (analysis of variance p = 0.02). Dunnett's post hoc test revealed lower biomass compared to control in the Guest River and Copper Creek treatments (exact p values shown). ART = Artrip; CAR = Carterton; CC = Copper Creek; CHP = Clinchport; CLE = Cleveland; DC = Dumps Creek; GR = Guest River; HF = Horton Ford; IN = Indian Creek; PEN = Pendleton; SAN = sand; SEM = Semones; SP = Spring River; WBS = West Bearskin.

the Clinch River treatments ranged from 1.5% to 4.4%, with no apparent pattern related to the sediments from the mussel zone of decline or to contaminant concentrations (Supplemental Data, Figure S1).

Overlying water ammonia

Median ammonia concentrations in overlying water ranged from 0.01 mg to 0.32 mg total ammonia nitrogen/L in control treatments and from 0.47 mg to 1.58 mg total ammonia nitrogen/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 compared with measurements at nearly every other time interval, indicating a skewed data set. Moreover, the median better reflected the long-term trend in exposure than did the mean. For context of the results, the 1-h (acute), 4-d, and 30-d (chronic) ammonia water quality values at the test temperature (20 °C) and average test pH of 8.43 are 1.8 mg, 1.0 mg, and 0.41 mg total ammonia nitrogen/L, respectively [33] (Figure 4).

Ammonia concentrations in overlying water were high in several treatments during week 1 of the experiment (mean 4.16 mg total ammonia nitrogen/L and range 0.71-6.26 mg total ammonia nitrogen/L in Clinch sediments; range <0.01–1.89 mg total ammonia nitrogen/L in controls) when the duration of contact with sediments was the longest (day -3 through day 3), followed by a decline in most treatments at the week-2 sampling point (day 10) after conducting 2 scheduled water renewals. However, ammonia remained high in the Guest River and Copper Creek treatments (3.13 mg and 2.0 mg total ammonia nitrogen/L, respectively) compared with others (range 0.02-1.24 mg total ammonia nitrogen/L in all other Clinch sediments, 0.01–0.62 mg total ammonia nitrogen/L in controls; Figure 4; Supplemental Data, Table S2). Week-1 ammonia concentrations exceeded the 1-h acute ammonia water quality criterion values (range 1.0–1.8 mg/L at respective mean treatment pHs) [33] in every Clinch treatment except Dumps Creek. In week 2,



Figure 4. Measured total ammonia nitrogen (TAN) concentrations in overlying water samples (1 composite sample/treatment) at week 1 (day 3, black bars) and week 2 (day 10, gray bars) during the 28-d sediment test with freshwater mussels. Horizontal lines indicate the 1-h, 4-d, and 30-d criteria (1.8 mg, 1.0 mg, and 0.41 mg TAN/L, respectively) at the test temperature (20 °C) and overall mean pH of 8.4. Although individual treatment pH varied slightly, the trend of criterion exceedances matches. Sites in the documented zone of mussel decline are denoted by the box. Note: The criteria and exceedances shown do not apply to the West Bearskin control treatment because of the comparatively lower pH (7.7). The 1-h, 4-d, and 30-d criteria at 20 °C for West Bearskin are 6.7 mg/L, 2.8 mg/L, and 1.1 mg/L, respectively. ART = Artrip; CAR = Carterton; CC = Copper Creek; CHP = Clinchport; CLE = Cleveland; DC = Dumps Creek; GR = Guest River; HF = Hoton Ford; IN = Indian Creek; PEN = Pendleton; SAN = sand; SEM = Semones; SP = Spring River.

ammonia in only the Guest River and Copper Creek treatments remained above the respective 1-h acute criteria; ammonia in the Indian Creek, Artrip, Cleveland, and Clinchport treatments exceeded the 4-d value (range 0.63-1.03 mg/L at test conditions). Ammonia in all other treatments was less than the respective 30-d chronic value (range 0.25-0.41 mg/L). In weeks 3 and 4, ammonia concentrations were <0.1 mg total ammonia nitrogen/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 1 and 2 treatments, respectively (Supplemental Data, Table S3). Metal

concentration differences between pretest and posttest samples and those in replicates with mussels versus empty replicates were minimal (Supplemental Data). Mean metal PEC quotients 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 (Table 1). Spring River and West Bearskin control sediments had mean PEC quotients of 0.08 and 0.14, respectively. A mean PEC quotient for the sand control was not calculated because concentrations were below detection limits for 8 of the 9 metals for which PECs are available; only Zn was present but at a very low concentration (1.20 µg/g dry wt, compared to the Zn PEC of 459 μ g/g). Individual PEC quotients 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 PEC quotients among treatments for As, Cr, Cu, Pb, and Zn were each <0.1; PEC quotients for Cd were not calculated because concentrations were below the detection limit (0.25 µg/g dry wt) for all treatments. Manganese had the highest PEC quotient 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 (Spring River = 0.13, West Bearskin = 0.12). Concentrations of Mn ranged from 197 µg/g to 630 µg/g dry weight in the collected sediments (Figure 5). The individual PEC quotient average for Fe (0.20) was also relatively high compared to other metals, though it fell between the control sediment values (Spring River = 0.10, West Bearskin = 0.30).

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 it was found only in the Guest River sediment at a very low concentration of 2.56 ng/g (dry wt), indicating 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; Supplemental Data, Table S4). Differences between pretest and posttest concentrations and between replicates with and without mussels were negligible (Supplemental Data). The PAH concentrations were >1000 ng/g in all Clinch River sediments, indicating widespread occurrence in the system. The West Bearskin control

 Table 1. Probable effect concentration (PEC) quotients (sediment concentration/PEC) of individual metals in each treatment for which PECs are available
 [30,31] and the mean metal PEC quotients for each sediment treatment^a

			Individual metal PEC quotients for each sediment treatment											
Metal	PEC	SP	WBS	IN	ART	CLE	DC ^b	CAR ^b	GR^{b}	SEM ^b	PEN ^b	CHP ^b	CC	HF
As	33	0.04	0.07	0.11	0.06	0.06	0.11	0.06	0.06	0.06	0.06	0.07	0.10	0.08
Cr	111	0.07	0.11	0.07	0.06	0.07	0.07	0.07	0.04	0.06	0.07	0.08	0.11	0.09
Cu	149	0.03	0.15	0.10	0.09	0.08	0.16	0.08	0.09	0.10	0.08	0.09	0.08	0.08
Fe	40 000	0.10	0.30	0.19	0.17	0.20	0.26	0.21	0.16	0.20	0.19	0.22	0.23	0.21
Mn	1100	0.13	0.12	0.18	0.20	0.32	0.38	0.27	0.57	0.32	0.35	0.39	0.49	0.33
Ni	48.6	0.10	0.26	0.11	0.10	0.12	0.16	0.12	0.17	0.13	0.13	0.15	0.13	0.12
Pb	128	0.06	0.04	0.05	0.06	0.07	0.07	0.07	0.06	0.07	0.06	0.07	0.08	0.06
Zn	459	0.12	0.08	0.06	0.07	0.07	0.13	0.07	0.09	0.07	0.06	0.07	0.06	0.06
Mean metal PEC quotient		0.08	0.14	0.11	0.10	0.12	0.17	0.12	0.15	0.13	0.13	0.14	0.16	0.13

^aMetal concentrations (μ g/g dry weight sediment) and PECs (mg/kg dry weight sediment) are in equivalent units.

^bClinch treatments from within the mussel zone of decline.

ART = Artrip; CAR = Carterton; CC = Copper Creek; CHP = Clinchport; CLE = Cleveland; DC = Dumps Creek; GR = Guest River; HF = Horton Ford; IN = Indian Creek; PEC = probable effect concentration; PEN = Pendleton; SEM = Semones; SP = Spring River; WBS = West Bearskin.



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

concentration of 1484 ng/g was mostly perylene, which is common in sediments with high organic content and associated with nonanthropogenic sources such as woody vegetation [35] and diatoms [36]. 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 [33] for a few individual PAHs in a few treatments, but only the Carterton treatment exceeded the PEC (22 800 ng/g) for total PAH. The PEC quotients 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 PEC quotients (0.65, 0.52, and 0.32, respectively). These PEC quotients were calculated using the sum of 42 PAHs, thus potentially overestimating risks compared with earlier studies that used fewer individual PAHs to estimate total PAH [20,31]. However, while using only the 13 PAHs reported recently by Wang et al. [20] lowers the PEC quotient values, it does not alter the overall conclusions because the Carterton site still exceeds the threshold of 1.0 (recalculated PEC quotient = 1.28). This illustrates an issue

with using the PEC approach for PAHs and is why we suggest using the sum equilibrium-partitioning sediment benchmark toxic unit approach as a more robust estimate of PAH risk. The more updated sum equilibrium-partitioning sediment benchmark toxic unit approach resulted in acute toxic unit values ranging from <0.01 to 0.37 and chronic toxic unit values ranging from 0.02 to 1.51. Only the Carterton site exceeded a chronic toxic unit of 1.0, but several sites were substantially elevated above the others (Figure 6B). These included Pendleton (0.21), Guest River (0.27), and Dumps Creek and Clinchport, where toxic unit values for both were above 0.40.

Most sediments were dominated by petrogenic PAHs (i.e., fossil fuel–sourced including most of the PAHs from unburned coal), accounting for 65% to 85% of total PAH compared to the pyrogenic portion (i.e., sourced from combustion/burning of fossil fuels) [37,38]. Petrogenic and pyrogenic PAHs were more evenly represented in Clinchport sediment (55% and 45%, respectively). Counter to most other treatments, pyrogenic PAHs dominated in Carterton and Copper Creek sediments, accounting for 71% and 64% (respectively) of all PAHs, whereas petrogenic PAHs contributed \leq 36%.



Figure 6. Total concentration of polycyclic aromatic hydrocarbons (PAH) (sum of all 42 measured) from sediment samples taken at the beginning of the 28-d test with freshwater mussels (A) and corresponding sum equilibrium-partitioning sediment benchmarks toxic units (ESBTU) for chronic toxicity (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 (gray bars = mainstem, hatched bars = tributary, arranged in order from upstream to downstream) sediments. Sites in the documented zone of mussel decline are denoted by the box. ART = Artrip; CAR = Carterton; CC = Copper Creek; CHP = Clinchport; CLE = Cleveland; DC = Dumps Creek; GR = Guest River; HF = Horton Ford; IN = Indian Creek; PEN = Pendleton; SEM = Semones; SP = Spring River; WBS = West Bearskin.



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

Passive sampling devices

Dissolved fraction PAHs were detected in overlying water by passive sampling devices in all treatments. There was a narrower range of PAH concentrations in the passive sampling devices compared to those in sediments, with total PAH ranging from approximately 1550 ng/L to 3350 ng/L. Corresponding chronic toxic unit values also had a narrower 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 passive sampling devices was consistent among exposures, with petrogenic PAHs comprising approximately 65% to 80% of the PAHs and pyrogenic PAHs 20% to 35%. Naphthalene was not detected in any of the passive sampling devices, indicating possible loss of more volatile PAHs from the water and that the concentrations of other more volatile PAHs may be low as well. Similar to results from the sediment analyses, few other organic contaminants were detected in the water. No PCBs or current-use pesticides were detected, and there were only 2 very low detections of chlordane-related organochlorine pesticides in the polyethylene passive sampling devices. Moreover, the 2 passive sampling device types provided a consistent indication of no or very low exposure to PCBs and pesticides in the water in all treatments.

Correlation analysis and explanatory statistical models

Only Mn and cobalt (Co) were negatively correlated with mussel survival and biomass, as was median ammonia concentration with survival and TOC with biomass (Figure 7 and Table 2). No other metals, water quality parameters, or sediment characteristics were negatively correlated with biotic endpoints (Supplemental Data, Table S5). Further, there were no significant correlations between mussel endpoints and TOC-normalized sediment concentrations of individual PAHs or total PAH acute or chronic toxic units (Supplemental Data, Table S5). Because Mn and Co covaried (p < 0.0001) 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 correlated variables, total PAH chronic toxic unit 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 data set 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 (equivalent AIC_c and model weight; 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

 Table 2. Pearson product-moment and Spearman rank-order correlation coefficients and p values between mussel survival and biomass and negatively correlated sediment treatment constituent concentrations from the 28-d mussel toxicity test

Parameter	Pearson	р	Spearman	р
Survival				
Cobalt	-0.58	0.04	-0.38	0.20
Manganese	-0.67	0.01	-0.27	0.38
Median ammonia	-0.81	< 0.01	-0.52	0.07
Biomass				
Cobalt	-0.62	0.02	-0.49	0.09
Manganese	-0.66	0.01	-0.42	0.16
Carbon (%)	-0.51	0.07	-0.63	0.02

total ammonia nitrogen. 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 total ammonia nitrogen 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; so on an environmental scale, a 100 µg/g increase in Mn resulted in a 4% reduction in survival (Table 3).

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; $r^2 = 0.39$). Manganese 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 model 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. Although sediment traps are regularly used in studies of erosion/sediment transport and particle settlement, plankton settlement, and even collection of sediments for contaminant analysis [39], the present study describes 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 allowed for evaluation of the toxicity of the suspended sediment load in near real time (within 5 mo of deposition). The present 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 [21] and the standard methods for evaluating sediment-associated contaminants with other fresh-water invertebrates [40].

Ammonia and Mn detrimentally affected mussel survival and biomass in the exposures to sediments from the Clinch River watershed, as demonstrated by quantitative explanatory statistical models. The most prevalent 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 is uncertain, but the finding is consistent with recent research that found that the carbon content of Clinch River bed sediments was negatively correlated with several mussel health metrics [9].

Although Guest River and Copper Creek were the most toxic sediments in the present study, the 2 watersheds have widely different land uses [9,10] (Figure 8). Copper Creek has been considered a candidate site for aquatic conservation [41] because it is 1 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) [9], unmined tributary watersheds like Copper Creek may be sourcing other pollutants (i.e., ammonia) to the Clinch River via its suspended sediment load.

Ammonia

Several studies report acute and chronic lethal and sublethal ammonia concentrations for unionid mussels of <1 mg total ammonia nitrogen/L [42–45], which is substantially less than some concentrations that we observed in the present study. While it is known that juvenile mussels may pedal feed below the surface [26] and thus may be less exposed to contaminants in

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

		stopes						
		Mo	del ranking		Partial slope p values			
		AIC _C	Δ_i	Wi	Ammonia	Mn	Carbon	
Candidate mod	lel selection							
Survival								
1	Ammonia	276.3102	0	0.42				
2	Ammonia + Mn	276.3875	0.08	0.41				
3	Ammonia + Mn + PAH chronic TU	278.1071	1.80	0.17				
Biomass								
1	Mn + carbon	314.0853	0	0.69				
2	Mn + carbon + PAH chronic TU	316.4301	2.34	0.21				
3	Mn + carbon + PAH chronic TU + ammonia	318.7827	4.70	0.07				
4	Mn	320.6459	6.56	0.03				
Most parsimor	ious models							
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	
Biomass	95.64–33.47 (Ammonia) 106.62–26.33 (Ammonia)–0.04 (Mn) 85.65–0.14 (Mn)–6.90 (carbon)				<0.01 <0.01	0.14 <0.01	<0	

Ammonia = median total ammonia nitrogen concentration; Mn = manganese concentration; carbon = sediment total organic percent; PAH chronic TU = equilibrium sediment-benchmark chronic toxic unit for total polycyclic aromatic hydrocarbons.



Figure 8. Land-cover/land-use categories in the Guest River (\mathbf{A}) and Copper Creek (\mathbf{B}) subwatersheds 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. (Land use and cover from the 2011 National Land Cover Database; watershed and stream features from the National Hydrography Dataset; surface mines available from Appalachian Voices (accessed 27 October 2015; Supplemental Data). Figure prepared by Steven Alexander, US Fish and Wildlife Service).

surface/overlying water, adult mussels and juveniles as small as 3 mm have been shown to siphon at the surface or sedimentwater interface [12,20]. Given the relatively high overlying water concentrations of total ammonia nitrogen measured in the present study, it is possible that the porewater ammonia could have been greater. However, toxicity is dependent on subsurface temperature and pH [33], which may have been lower just below the sediment surface, thus reducing toxicity [43]. At least 1 study of bed sediments in a river found that porewater ammonia concentrations were significantly higher than those found in surface water samples and that sediment porewater ammonia may also be temporally more variable, having greater concentrations than surface waters in warmer months when higher temperatures increase toxicity [46]. Another study found that excessive interstitial ammonia in rivers was strongly linked to failures in mussel recruitment [47].

Though surface water ammonia concentrations in the Clinch River basin have steadily declined over the last few decades as a result of improving wastewater-treatment practices [8,9], the present findings indicate that suspended sediments, especially in the Guest River and Copper Creek, may be supplying ammonia (or constituents that enhance ammonia production) to the system. While we acknowledge that the ammonia concentrations observed in our staticrenewal test are likely greater than would occur under typical riverine conditions (i.e., flowing water with frequent flushing), we have potentially identified ammonia as being transported with suspended sediments entering the Clinch River. Although ammonia from human-related effluents has been reduced through improved treatment [4,8,9], other sources, such as livestock and other agricultural runoff, may be contributing [41,48]. Nearly 41% of land use in the Copper Creek watershed is classified as hay/pasture (S. Alexander, US Fish and Wildlife Service, Cookeville, TN, personal communication). Though more than half of the Copper Creek watershed is forested, riparian forest is limited [41]. Pastures line the banks, livestock is abundant, and cattle have direct access to the stream [41]. 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 [41]. Improved land management may help reduce sedimentation and diminish potential risks to freshwater mussels and other taxa.

Metals

Manganese 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 [49]. After 28 d of exposure, Mn and Co concentrations were the only sediment metal parameters that correlated with mussel survival and biomass, and subsequent explanatory models that included Mn partially explained the variation in mussel biomass, especially. Both Mn and Co can be associated with rejected spoil rock from coal-mining operations [50]. Johnson et al. [9] 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 population metrics such as recruitment and number of imperiled species. Most coal-mining and, therefore, spoil rock sources of Mn are limited to the Appalachian Plateau on the northern side of the Clinch River basin (e.g., Guest River; Figure 8) [4]. There is no known coal mining in the Copper Creek watershed (Figure 8), and the source of Mn there is uncertain at this time. However, the limestone geological formations in the valley and ridge region can be naturally rich with Mn deposits [51,52].

Although mean metal PEC quotients for each treatment were substantially less than the established toxicity benchmark of 1.0 for sediment quality, other investigators have found sediments with mean PEC quotients <0.4 to be toxic to freshwater mussels, including some with PEC values in the same range as the present results [20]. As previously stated, the toxicity benchmarks were established using toxicity data primarily from other classes of invertebrates (e.g., midges and amphipods) [31]. Although Mn was not among the sediment metals included by Wang et al. [20], their results suggest that established PECs may not be representative of contaminant risks to freshwater mussels.

Organics

There was an overall lack of organochlorine and current-use pesticides and PCBs measured in the sediment deposited and tested during the present study. However, collection of riverine suspended sediments during spring when the majority of pesticides are likely applied, as opposed to our late summer/ autumn collection, would be appropriate for future study. The most abundant class of organic contaminants measured was the PAHs. The sediment at the Carterton site had the greatest PAH content compared to other Clinch River mainstem and tributary sediments. The prevalence of pyrogenic PAHs at Carterton is likely a result of its close proximity to (and location downstream of) a coal-fired power plant along the Clinch River at Carbo (VA, USA). 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 [53]. The Guest River and Dumps Creek tributaries also transport PAHs. The Guest River had total PAH concentrations greater 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 among the top 3 PAH chronic toxic unit scores, surpassing those of the Guest River.

Despite the lack of statistical relation of PAHs to mussel survival and biomass in the 28-d test, our analyses demonstrate that the sediment load contributes substantially to the influx of PAHs within the Clinch River and its tributaries. Considering the high PAH concentration and chronic toxic units at Carterton and the elevated toxic units at Clinchport, Dumps Creek, and other sites within the mussel zone of decline, long-term exposure to these PAHs may be adversely affecting mussels and perhaps other aquatic life. The 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., up-regulated biomarker enzymes, differential tissue concentrations) [54] that may be addressed in future studies with juvenile mussels. Such effects may impact immunity, reproduction, or other processes that can lead to longterm population-level declines, such as 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 cotoxic 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 [55].

IMPLICATIONS AND CONCLUSIONS

The responses of juvenile mussel survival and biomass were associated with ammonia and Mn measured in the suspended sediment load collected with sediment traps from the Clinch River and especially its tributaries, Guest River and Copper Creek. Long-term exposures to such conditions at these sites could be problematic for juvenile and adult mussels. In addition, fully chronic exposures (over months or years compared to our 28-d test) could allow time for other contaminants, such as the prevalent PAHs, to adversely affect mussel biomass and survival. These contaminants are already known to alter aquatic fauna at subcellular scales (e.g., carcinogenesis, teratogenesis) [54,56] and, thus, may impact immunity and fecundity in mussels, in addition to the more coarse-scale endpoints examined in the present study. This sediment-load burden of PAHs, ammonia, Mn, and elevated concentrations of other metals demonstrates influx and transport within the basin and may include naturally occurring and anthropogenic sources of 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 that negative synergistic effects are the most likely outcome [55]. Future studies similar to the present study may consider alternate designs with more replication or more frequent assessment of endpoints (may require destructive samples) to quantify interactive effects and evaluate subcellular endpoints. Finally, the suspended sediment load is an important source of contaminants that should be considered. The present results 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 suspended sediment load and improving the overall health of the Clinch River ecosystem.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3540.

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Data availability—Data, associated metadata, and calculation tools may be obtained by contacting the corresponding author (jmarcham@ncsu.edu).

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