Published in final edited form as:

Environ Toxicol Chem. 2017 February ; 36(2): 395–407. doi:10.1002/etc.3540.

Assessing toxicity of contaminants in riverine suspended sediments to freshwater mussels

Jennifer M. Archambault^{*,†}, Christine M. Bergeron[†], W. Gregory Cope[†], Peter R. Lazaro[‡], Jeremy A. Leonard[†], and Damian Shea[‡]

[†]Department of Applied Ecology, North Carolina State University, Raleigh, North Carolina, USA

[‡]Environmental Chemistry and Toxicology Laboratory, North Carolina State University, Raleigh, North Carolina, USA

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 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 and organic 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. We 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.

Keywords

Unionoida; Water quality; Ammonia; Metals; Polycyclic aromatic hydrocarbons (PAHs)

*Corresponding author: Jennifer M. Archambault – Department of Applied Ecology, North Carolina State University, Campus Box 7617, Raleigh, NC, USA; telephone (919) 306-5107; fax (919) 515-4454; jmarcham@ncsu.edu.

SUPPLEMENTAL DATA

Metal and organic quality control measures.

Contaminant comparisons pre- vs. post-test and mussel vs. empty replicates.

Geospatial layer metadata for Figure 8.

Figure S1.

Tables S1 – S5.

INTRODUCTION

The southeastern United States (US) 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, ~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 years [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 over-arching theme. Analyses of datasets 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 – 1980s, though surface water concentrations have improved considerably, especially in the most recent decade [8]. Metal concentrations, including those released in two 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–1990s and have declined over time [6,8], but some metals may still be a cause of concern (e.g., Fe and 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].

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 [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 of 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 non-point 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. While 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 this 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.

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 ($n = 3$) 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), Semones (SEM), Pendleton (PEN), and Clinchport (CHP), are within the zone of decline; and Copper Creek (CC; a tributary), 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 Valley and Ridge physiographic landforms, while Indian and Dumps Creeks and the Guest River enter from the northwest, draining the coal- and gas-rich 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 PVC 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 months (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 ~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 (EPA) standards for metal, pesticide, and semi-volatile analysis (Thermo Scientific ICHM 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, sampled for particle size, total organic carbon content characterization, and contaminant analysis [15]. Afterward, they were returned to the refrigerator for a total holding time of two weeks 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 static-renewal 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 vs. leaching into the dissolved fraction of overlying water. Polyethylene and universal passive sampling devices (PSDs) [17] were randomly placed into the overlying water of 1 replicate in 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 (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 (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 [18–20]. The third control sediment was a commercially available, contaminant-free filter sand (SAN) (Southern Products and Silica Co., Inc.), which has been used in previous toxicological studies with freshwater mussels [12].

The test was conducted in light- and temperature-controlled environmental chambers (Precision Model 818, Thermo Fisher Scientific), 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 [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 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 – 209 mg CaCO₃/L alkalinity, 156 – 200 mg CaCO₃/L hardness, 520 – 578 µS/cm conductivity, 7.72 – 8.69 pH, and 6.98 – 8.89 mg/L dissolved oxygen (n = 15). Additionally, overlying water samples for total ammonia nitrogen (TAN) analysis were collected weekly from all treatments to ensure feeding (see below) 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, North Carolina, 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 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 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 due in large part to their unique parasitic (upon a host fish) and multi-stage life cycle. 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 this 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, Virginia, 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, upon arrival, were slowly acclimated from culture water to test water and to 20°C following American Society for Testing and Materials 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 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 SiO₂ 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 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, approximately half of them 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 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 [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 Corporation) for determination of biomass (total dry weight 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, 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.). 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 (SAN, SP, WBS; $\alpha = 0.10$, exact p-values reported). 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.

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 (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) [30,31]. 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. 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 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 [32]. 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) [32] to calculate the equilibrium-partitioning sediment benchmark toxic unit (ESBTU). The individual PAH ESBTUs were totaled; the resulting sum ESBTU (Σ ESBTU, hereafter 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 to provide comparison to previous studies that have used that approach.

Measured TAN 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 TAN results are reported and discussed for comparison to the current ammonia criteria, which include acute and chronic values 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 [33]. 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 $2.5 \times$ the chronic criterion more than once in three years [33]. 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 ($2.5 \times$ the chronic criterion) under the same conditions.

To gain a better understanding of the variables potentially influencing the significant effects of sediment treatments on 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 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 extreme values. Correlated variables 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 the affected mussel endpoints were selected from all possible 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 mg (± 0.219 mg, SE). 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).

Percent 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 percent increase was in the sand control (1.3%). Mussel length measurements in the Clinch River treatments ranged 1.5 – 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 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 compared to measurements at nearly every other time interval, indicating a skewed dataset. 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) ammonia water quality values 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 [33] (Figure 4).

Ammonia concentrations in overlying water were 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 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 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; Supplemental Data, Table S2). Week 1 ammonia concentrations exceeded the 1-hr 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, 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 exceeded the 4-day value (range 0.63 – 1.03 mg/L at test conditions). Ammonia in all other treatments was less than the respective 30-day chronic value (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 1 and 2 treatments, respectively (Supplemental Data, Table S3). Metal concentration differences between pre- vs. post-test samples and those in replicates with mussels vs. empty replicates were minimal (Supplemental Data). 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 (Table 1). 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 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 weight, 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 dry weight) 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 dry weight in the collected sediments (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).

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 weight), 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 pre- vs. post-test concentrations and between replicates with and without mussels were negligible (Supplemental Data). PAH concentrations were > 1000 ng/g in all Clinch River sediments, indicating widespread occurrence in 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 [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. 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). These PECQs were calculated using the sum of 42 PAH, thus potentially overestimating risks compared to 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 PECQ values, it does not alter the overall conclusions because the Carterton site still exceeds the threshold of 1.0 (recalculated PECQ = 1.28). This illustrates an issue with using the PEC approach for PAHs and is why we suggest using the ΣESBTU approach as a more robust estimate of PAH risk. The more updated ΣESBTU approach resulted in acute TU values ranging < 0.01 – 0.37 and chronic TU values ranging 0.02 – 1.51. Only the Carterton site exceeded a chronic TU of 1.0, but several sites were substantially elevated above the others (Figure 6B). These included Pendleton (0.21) and Guest River (0.27), and 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) [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 36%.

Passive sampling devices

Dissolved fraction PAHs were detected in overlying water by PSDs in all treatments. There was a narrower 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 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 PSDs was consistent among exposures, with petrogenic PAHs comprising about 65 – 80% of the PAHs, and pyrogenic PAHs 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 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 PSDs. Moreover, the two PSD 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 Co were negatively 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 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 TUs (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 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 (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; 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 ($F_{2,36} = 11.44$, $p < 0.01$; $r^2 = 0.39$). 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 [39], 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 allowed for the evaluation of the toxicity of the suspended sediment load in near real-time (within 5 months of deposition). 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 [21] and the standard methods for evaluating sediment-associated contaminants with other freshwater invertebrates [40].

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 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 the carbon content of Clinch River bed sediments was negatively correlated with several mussel health metrics [9].

Though Guest River and Copper Creek were the most toxic sediments in our study, the 2 watersheds have widely different land uses [9,10] (Figure 8). Copper Creek has been considered as a candidate site for aquatic conservation [41] 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) [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 TAN/L [42–45], which is substantially less than some concentrations that we observed in this study. While it is known that juvenile mussels may pedal feed below the surface [26] and thus may be less exposed to contaminants in surface/overlying water, adult mussels and juveniles as small as 3 mm have been shown to siphon at the surface or sediment/water interface [12,20]. Given the relatively high overlying water concentrations of TAN measured in this study, it is possible that the pore-water 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 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 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 due to improving wastewater treatment practices [8,9], our 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 static-renewal 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. While ammonia from human-related effluents have been reduced through improved treatment [4,8,9], other sources, such as livestock and other agricultural runoff may be contributing sources [41,48]. Nearly 41% of land use in the Copper Creek watershed is classified as hay/pasture (S. Alexander, US Fish and Wildlife Service, 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

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 [49]. After 28 days 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. 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 Plateaus 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].

While mean metal PECQs for each treatment were substantially less than 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 [20]. As previously stated, the toxicity benchmarks were established using toxicity data primarily from other classes of invertebrates (e.g., midges and amphipods) [31]. Though 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 our 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. PAHs were the most abundant class of organic contaminants measured. 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 due to its close proximity to (and downstream of) a coal-fired power plant along the Clinch River at Carbo (Virginia, 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 are also transporting 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 three PAH chronic TU scores, surpassing those of the Guest River.

Despite the lack of statistical relation of PAHs to mussel survival and biomass in our 28-day 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 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 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., upregulated 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 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 [55].

IMPLICATIONS/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/or adult mussels. Additionally, fully chronic exposures (over months or years, compared to our 28-day test) could allow time for other contaminants, like the prevalent PAHs, to adversely affect mussel biomass and survival. These contaminants are already known to alter aquatic fauna at sub-cellular 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 here. 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 negative synergistic effects are the most likely outcome [55]. Future studies similar to this one may consider alternate designs with more replication or more frequent assessment of endpoints (may require destructive samples) to quantify interactive effects and evaluate sub-cellular endpoints. Finally, the suspended sediment load is an important source of contaminants that should be considered. 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 suspended sediment load and improving the overall health of the Clinch River ecosystem.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding for this research was provided by the US Geological Survey (USGS) and US Fish and Wildlife Service (USFWS) through the Science Support Partnership Program via Research Work Order No. 197, administered through the USGS NC and VA Cooperative Fish and Wildlife Research Units, and by National Institute of Environmental Health Sciences (Grant P42 ES005948). F. Weber (RTI International, Research Triangle Park, NC) analyzed metals; C. Niewoehner, (Department of Soil Science, NC State University, Raleigh, NC) performed particle size analysis; L. Mackenzie, J. James, and staff at the Center for Applied Aquatic Ecology (NC State University) analyzed ammonia content of water samples. M. Bradley (Virginia Department of Game and Inland Fisheries, Marion, VA) provided juvenile mussels. J. Smith (Department of Statistics, NC State University)

provided statistical advice and expertise. S. Alexander (USFWS, Tennessee Ecological Services Field Office, Cookeville, TN) prepared map images for Figure 8. N. Wang and C. Ingersoll (USGS, Columbia Environmental Research Center, Columbia, MO) provided advice on test methods and procedures. A. White and B. Cope constructed and assembled the sediment traps. Finally, we are grateful for the input and local expertise of several collaborating researchers including B. Beaty, J. Jones, B. Evans, B. Kreps, S. Ciparis, and M. Schreiber.

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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 sites (triangles). The zone of documented mussel decline is denoted by the red box and includes Dumps Creek, Carterton, Guest River, Semones, Pendleton, and Clinchport.

Figure 2.

Mean survival ($\pm$ 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; Semones, SEM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Sites in the documented zone of mussel decline are denoted by the red box. There was a significant effect of sediment treatment on survival (ANOVA $p < 0.01$). A Dunnett's post-hoc test revealed lower survival compared to control in the Guest River and Copper Creek treatments (exact p-values shown).

Figure 3.

Mean change in biomass ($\pm$ 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; Semones, SEM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Sites in the documented zone of mussel decline are denoted by the red 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 lower biomass compared to control in the Guest River and Copper Creek treatments (exact p-values shown).

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; Semones, SEM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Sites in the documented zone of mussel decline are denoted by the red box.

Note: The criteria and exceedances shown do not apply to the West Bearskin control (WBS) treatment because of 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.

A. Mn concentration

B. Mn PEC quotient

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; West Bearskin, WBS; Indian Creek, IN; Artrip, ART; Cleveland, CLE; Dumps Creek, DC; Carterton, CAR; Guest River, GR; Semones, SEM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Sites in the documented zone of mussel decline are denoted by the red box.

A. Total PAH

B. PAH chronic toxic units

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 ΣESBTUs) (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; Semones, SEM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Sites in the documented zone of mussel decline are denoted by the red box.

A. Mn vs. Survival **C. Ammonia vs. Survival** **B. Mn vs. Biomass** **D. Carbon vs. Biomass** **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.

A. Guest River watershed **B. Copper Creek watershed** **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. (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 (Boone, NC); accessed 27 October 2015. Figure prepared by Steven Alexander, US Fish and Wildlife Service. (Supplemental Data))

Table 1

Probable effect concentration quotients (PECQs) of individual metals in each treatment for which Probable Effect Concentrations (PECs) are available [30,31], and the mean metal PECQ for each sediment treatment. Metal concentrations ($\mu\text{g/g}$ dry weight sediment) and PECs (mg/kg dry weight sediment) are in equivalent units; $\text{PECQ} = \text{sediment concentration}/\text{PEC}$.

Metal	PEC	Individual Metal PECQs for each Sediment Treatment												
		SP	WBS	IN	ART	CLE	DC	CAR	GR	SEM	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

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; Semones, SEM; Pendleton, PEN; Clinchport, CHP; Copper Creek, CC; and Horton Ford, HF. Clinch treatments shaded are those from within the mussel zone of decline.

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-day mussel toxicity test.

Parameter	Survival			
	Pearson	p-value	Spearman	p-value
Co	−0.58	0.04	−0.38	0.20
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
Co	−0.62	0.02	−0.49	0.09
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.

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 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 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

Ammonia = median total ammonia nitrogen concentration, Mn = manganese concentration, Carbon = sediment total organic percent, and PAH Chronic TU = equilibrium sediment-benchmark chronic toxic unit for Total PAH.