Evaluation of hollow fill drainages and associated settling ponds on water quality and benthic macroinvertebrate communities of VA and WV.

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Abstract

Hollow fill drainages and settling ponds, products of surface coal mining, were evaluated in Virginia and West Virginia for potential influences upon water quality and benthic macroinvertebrate communities of low order streams. Sampling sites were located in the South Fork of the Pound River and Powell River watersheds (Wise County) in Virginia and Lavender Fork (Boone County), Five Mile Creek (Mingo County), and Trace Fork (Mingo County) in West Virginia. Bioassessment techniques, including water column toxicity testing with *Ceriodaphnia dubia*, sediment toxicity testing with *Daphnia magna*, *in situ* Asian clam (*Corbicula fluminea*) toxicity testing, and benthic macroinvertebrate analysis, were utilized in evaluating the environmental influences originating from the hollow fills and the potential impact upon the aquatic receiving systems. Hollow fill drainages had higher conductivity compared to mainstem and sites below settling ponds. Benthic macroinvertebrate richness levels were reduced in fill drainages compared to mainstem sites, which can be attributed to poor habitat conditions in the drainages. Population composition analyses of %*Ephemeroptera* and %*Chironomidae* suggested that fill drainages were comprised of a more tolerant community compared to mainstem sites and sites downstream of the settling ponds. Asian clam growth and benthic macroinvertebrate collector filterers were significantly higher at sites in close proximity to the settling ponds. The enhanced collector filterer populations could be attributed to potential organic enrichment originating from the settling ponds.

1. Introduction

Coal mining in the Appalachian region utilizes a method of spoil disposal known as head of hollow filling. Essentially, the excess spoil and material generated from surface coal mining is disposed in hollows near or adjacent to the mined land area. The use of hollow fills is an environmentally sensitive topic due to the potential influences originating from these fills, as well as associated drainages and settling ponds, and the possible effects upon low order stream water quality and aquatic life. Headwater streams, which are ecologically significant areas in aquatic systems, typically inhabit specialized taxa and have intimate interactions with their riparian surroundings; (i.e. leaf litter and woody debris processing). Assessments of potential hollow fill drainage influences, if any, upon headwater streams is essential to upholding water quality standards in coal mining regions.

Disturbances to aquatic systems associated with mine drainages have been studied in a number of streams in the Appalachian region of the United States (Nelson and Roline, 1996; Schultheis et al. 1997; Cherry et al. 2001; Soucek et al. 2001; Kennedy et al. 2003). Some severe mine drainages have caused stream acidification, which has been found to significantly impair benthic macroinvertebrates with the loss of sensitive species such as *Ephemeroptera* and several bivalve species (Leland et al. 1989; Clements, 1994; Courtney and Clements, 1998; Guerold et al. 2000). Additional perturbations to aquatic systems associated with severe mine drainages may include sedimentation of stream habitat (Wood and Armitage, 1997; Bonta, 2000), heavy metal influx and precipitation resulting in shifts in the benthic communities from sensitive to more tolerant taxa (Clements, 1994; Beltman et al. 1999; May et al. 2001), and acid mine drainage (Cherry et al. 2001; Soucek et al. 2001, 2002a, b, 2003; Schmidt et al 2002a, b).

The major objective of this study focused upon the potential influences originating from drainages of head of hollow fills generated from surface coal mining and associated settling ponds and the subsequent influences upon low order streams within Virginia and West Virginia. Physiochemical and biological parameters were measured to evaluate any potential negative influences that could hinder water quality or aquatic life of the low order streams. Bioassessment procedures utilized in the study included water/sediment analysis, water column/sediment toxicity testing with *Ceriodaphnia dubia* and *Daphnia magna*, benthic macroinvertebrate surveys, and *in situ* toxicity testing with Asian clams (*Corbicula fluminea* [Müller]).

2. Methods

2.1 Sampling Sites

Eight hollow fills were evaluated in five different watersheds within the Appalachian region of southern Virginia and West Virginia, USA (Fig. 1). Thirty two sampling locations were utilized in the study, consisting of reference sites, hollow fill drainages, and areas below settlings ponds and in the mainstem receiving systems. Two watersheds located in Virginia were sampled to evaluate four fills; including the South Fork of the Pound River and the Powell River watersheds in Wise County. These watersheds and study areas in Virginia consisted of fill drainages entering into established streams, meaning the headwaters of the receiving systems did not originate from the hollow fill drainages. Three watersheds in West Virginia were sampled evaluating four hollow fill drainages and sites below settling ponds; including one in Lavender Fork in Boone County, and one in Five Mile Creek and two in Trace Fork in Mingo County. These watersheds and study areas in West Virginia consisted of fill drainages and settling ponds that were the direct origins of the stream systems. Sampling sites and conditions are summarized in Tables 1a and 1b.

Two hollow fills each were evaluated in the South Fork of the Pound River (SFPR) and Powell River watersheds in Wise County, VA. The two fills assessed in the SFPR watershed were approximately ½ stream mile apart from one another discharging into the SFPR mainstem. The uppermost hollow fill was 11 yrs. old (time since completion), with a volume of 420,899 cubic yards and vegetation limited to grass and small shrubs. The upper hollow fill drained into a series of two settling ponds before discharging into a small tributary leading to the SFPR, which subsequently was influenced by a nearby AMD seepage. The lower hollow fill, which was 12 yrs. old, had a volume of 156,029 cubic yards with grass as the dominate vegetation and was located approximately ½ mile downstream from the upper fill. Sampling sites were located above the settling ponds in the drainages of the two hollow fills and in the settling pond drainages before mixing with the SFPR mainstem. Additionally, sites were located in the SFPR mainstem directly above and below the confluence of the settling pond drainages. All sites utilized in the Powell River watershed were located in areas where the nearby terrain was heavily influenced by surface coal mining. A reference site was located in the receiving system above any mining influence related to the study. The first fill downstream of the reference site was approximately 16 yrs. old, with a volume of 162,554 cubic yards and well established vegetation and tree cover. The hollow fill did not have a consistent drainage, thereby mainly influencing the receiving system under rain events and potential subsurface drainages.

The second hollow fill was 7 yrs. old with a volume of 400,017 cubic yards, with grass as the dominant vegetation. Sampling sites were established in the fill drainage above the settling pond, and in the pond's drainage that discharged into the Powell River watershed. Sampling sites were located in the mainstem above and below the confluence of the second fill's pond discharge. It should be noted that within the mainstem potential influence from AMD was evident from small seepages within the stream. Sampling sites and conditions for the Virginia sampling areas are summarized in Table 1a.

The three watersheds sampled in West Virginia were sampled in the same manner due to similar conditions. In each watershed, sampling occurred at a drainage originating from the hollow fill flowing into a settling pond at the base of the fill. Next, there were sampling sites downstream of the settling ponds at approximately 100-150 m. increments in each watershed. There were five sampling sites established in the Five Mile Creek watershed in Mingo County, West Virginia. The hollow fill was completed 7 years ago (1995) with a volume of 122,100 cubic yards and grass as the dominant terrestrial vegetation including scattered shrubs and small trees. The sampling sites in Five Mile Creek consisted of one hollow fill drainage and three sites downstream of the settling pond. Additionally, a small tributary above any hollow fill or mining influence was established in the Five Mile Creek watershed as a regional reference site.

Seven sampling sites were established in the Trace Fork watershed, in Mingo County, assessing two hollow fill drainages and ponds. One branch of Trace Fork was influenced by a 3-year old hollow fill (1999) with a volume of 317,504 cubic yards and limited vegetation, consisting of mainly grass. A second branch of Trace Fork was influenced by a 15-year old hollow fill completed in (1987). The older hollow fill had a volume of 64,312 cubic yards with well established grass, tree, and shrub vegetation. Study sites for Trace Fork included a fill drainage site below each hollow fill and three sites below the younger fill's settling pond and two sites below the older fill's settling pond. Lastly, five sampling sites were established in the Lavender Fork watershed in Boone County, West Virginia. The hollow fill here was 6 years old (1996) with a volume of 115,840 cubic yards with grass as the dominant vegetation including established shrubs and small trees. One site was established in each of the two drainages originating from the fill above the settling pond, with three sites located downstream of the pond. Sampling sites and conditions for the West Virginia study areas are summarized in Table 1b.

2.2 Water Column & Sediment Chemistry

Water samples were collected and tested seasonally (winter, spring, summer, fall; n = 5) in 2002, with water quality parameters measured on each trip. Conductivity and pH measurements were conducted under field conditions and the samples were returned at 4°C where alkalinity and hardness were measured in the laboratory at Virginia Tech. The pH was measured using a Accumet ® (Fisher Scientific, Pittsburgh, PA, USA) pH meter equipped with an Accumet gel filled combination electrode (accuracy < ± 0.05 pH at 25°C). Conductivity measurements were made with a YSI (Yellow Springs Instruments, Dayton, Ohio, USA) conductivity meter, model 30/10. Alkalinity and hardness were measured by titration according to standard protocols (APHA 1995). Additionally, water and sediment samples were collected on one occasion for metals analysis by the Inductively Coupled Plasma Spectrometry (ICP) lab at Virginia Tech (not reported). Water samples were filtered (pore size 0.47 μm) and analyzed for dissolved aluminum (Al), copper (Cu), iron (Fe), and manganese (Mn) concentrations. Sediment samples were digested using (1+1) nitric acid and (1+4) hydrochloric acid under reflux heating according to U.S. EPA protocols (1991). Samples were then submitted to the ICP laboratory for analysis for total Al, Cu, Fe, and Mn concentrations.

2.3 Water Column Toxicity Testing

Water samples were collected seasonally in 1-L high density polyethylene bottles for toxicity testing of the cladoceran, *Ceriodaphnia dubia*. *Ceriodaphnia* were culture at Virginia Tech in U. S. EPA standard moderately hard synthetic (MHS) water (U.S. EPA 1993). Acute toxicity testing was a 0.5 serial dilution of the collected site water with U.S. EPA synthetic water as the diluent. Test organisms, < 24 hrs old, were used in the dilution series with four replicates, containing five *C. dubia* each, in 50-ml beakers. The testing occurred under static/nonrenewal conditions for 48 hrs in an incubator at 25±1°C with no feeding regime. Survival was recorded at 24 and 48-hr. intervals with a LC_{50} endpoint generated from the data using LC50/TOXSTAT software (Gulley 1996).

2.4 Sediment Toxicity Testing

Sediment samples were collected at the 17 sites (collected at the same time as sediment for metal analysis) in August 2002 for chronic toxicity testing using the cladoceran, *Daphnia magna*. *Daphnia* were cultured at Virginia Tech in 250-ml beakers containing three individuals with filtered Sinking Creek culture water, which had an average pH, conductivity, alkalinity, and hardness of 8.27 ± 0.11, 278 ± 14.9 μS/cm, 148 ± 7.44 mg/L as $CaCO_3$, and 148.9 ± 8.77 mg/L as $CaCO_3$, respectively. Sinking Creek is a reference stream in Newport, VA near Virginia Tech, Blacksburg, VA. Sediment samples were collected using a clean polypropylene scoop per site and placed in Ziploc® freezer bags, and stored at 4 °C for no more than two weeks. Tests were conducted according to procedures outlined by the American Society of Testing and Materials (ASTM, 1995) with modifications, using filtered Sinking Creek water as the overlying water. Four replications, each containing three *D. magna*, were used at each research site. The organisms were fed a diet of a 50/50 mixture of *Selenastrum capricornutum* and YCT (yeast, cerophyll, and trout chow). Each day of the 10-day test, survival and neonate production were recorded. The overlying water was renewed daily with aerated reference water followed by the daily feeding regime. Sediments from the reference sites were used as controls for the tests.

2.5 Benthic Macroinvertebrate Survey

Benthic macroinvertebrate surveys were conducted in accordance to the U.S. EPA Rapid Bioassessment Protocols (RBPs) (Barbour et al. 1999). A D-frame dipnet, with a 800-μm mesh net, was used to collect qualitative samples for each site. Four, three-minute, replications occurred in riffle, run, pool, and shoreline habitats. Samples were placed into plastic jars and preserved with 95% ethanol and returned to Virginia Tech for processing and identification. Organisms were identified to the lowest practical taxonomic level, normally genus (except *Chironomidae*), using standard identification keys and manuals (Merritt and Cummings, 1996; Pennak, 1989). Multiple community indices were calculated which included total taxa richness, *Ephemeroptera-Plecoptera-Trichoptera* (EPT) richness, %EPT, %*Ephemeroptera*, %*Chironomidae*, and functional feeding group compositions including shredders and collector filterers (not reported).

2.6 Habitat Assessment

Habitat conditions were assessed at each site using the U.S. EPA RBPs (Barbour et al. 1999). Parameters such as cobble size, stream width, riparian zone, and flow were measured on a rating scale of either 0-10 or 0-20, with a maximum score of 200. Therefore, the higher the score at a site, the better the habitat conditions. Two individual researchers conducted the habitat assessment surveys separately and mean scores for each site were recorded and utilized in statistical analyzes. Habitat assessment was important in determining whether landuse, shoreline degradation, or poor habitat conditions were responsible for any measured stress within the watershed at each sampling site.

2.7 *In situ* Asian clam toxicity testing

Asian clams collected from reference sites in the New River near Ripplemead, VA using clams rakes, were returned to the Ecosystem Simulation Laboratory (ESL) at Virginia Tech and stored in Living Streams® (Frigid Units, Toledo, OH, USA). Clams were measured using ProMax Fowler NSK calipers (Fowler Co. Inc., Boston, MA, USA) and individuals between 9.0 mm and 13.0 mm were distinctly marked with a file for later identification. Five clams were placed in polyurethane mesh bags (18 X 36 cm) with openings of 0.5 cm^2, and transported to the research sites. Four replicates (20 clams) were fastened and arranged around a rebar and placed in riffles at each research site. Growth and survival were measured every 30 days for a 60-day test period (May 15 thru

July 16, i.e. 62 days). Asian clam mortality was assessed as individuals with valves either gapping open or easily teased apart.

2.8 Statistical Analyses

Data including water quality analysis, sediment toxicity testing, macroinvertebrate indices, Asian clam endpoints, and habitat assessment were analyzed in the JMP IN® software (Sall and Lehman, 1996). Data were tested for normality using the Shapiro-Wilks test ($\alpha = 0.05$). Comparisons of normally and non-normal distributed data were made between the sites using the Tukey-Kramer honestly significant difference post-hoc test ($\alpha = 0.05$) and Wilcoxon Rank sum. Correlation analysis, using Pearson correlation for normal data and Spearman's analysis for non-normal data, of the biotic data against the abiotic parameters was conducted with site means in JMP IN®.

3. Results

3.1 Water Column and Sediment Chemistry

The greatest fluctuations in water quality perimeters for all Virginia/West Virginia watersheds were conductivity and water hardness. The fill drainages in the SFPR and Powell River watersheds, in Virginia, had the overall highest conductivity and water hardness, excluding an AMD influenced tributary. In the SPFR, fill drainages PdR-D1 and PdR-D2 had conductivity of 2872 ± 173 µS/cm and 2245 ± 311 µS/cm, respectively (Table 2a). Conductivity in the SFPR mainstem sites was significantly lower ($p < 0.05$) than the fill drainages, ranging between 1631 ± 210 µS/cm and 1657 ± 181 µS/cm at PdR4 and PdR2, respectively. The fill drainage, PR-D, in the Powell River watershed, had significantly higher ($p < 0.05$) conductivity of 1748 ± 207 µS/cm than all other sampling sites in the Powell River watershed. The only site with consistent acidic conditions was PdR-AMD, with 4.43 ± 0.4, which was not due to any hollow fill influence. Water hardness was closely related to conductivity ($p < 0.001$) with PdR-D1, PdR-D2 and PR-D significantly higher than mainstem sites in the respective streams with values of 2017 ± 281 mg/L as $CaCO_3$, 1350 ± 121 mg/L as $CaCO_3$, and 1050 ± 144 mg/L as $CaCO_3$, respectively.

In West Virginia, the water chemistry reported for sampling sites in the same watersheds had fewer significant differences among fill drainages and sites below settling ponds (Table 2b). However, the fill drainage, FMC-D, in Five Mile Creek, did have significantly higher ($p < 0.05$) conductivity, alkalinity, and water hardness as well as significantly lower ($p < 0.05$) pH with values of 1557 ± 544 µS/cm, 328 ± 88 mg/L as $CaCO_3$, 993 ± 343 mg/L as $CaCO_3$, and 7.42 ± 0.20, respectively, compared to sites below the settling pond. Water quality parameters for sites below the pond in Five Mile Creek were not significantly different from one another. The two branches of Trace Fork had limited significant differences between their fill drainages and sites below the ponds concerning conductivity. The older fill drainage in Trace Fork, TF-D2, had the highest conductivity, alkalinity, and water hardness in the watershed with values of 1636 ± 214 µS/cm, 194 ± 17 mg/L as $CaCO_3$, and 919 ± 102 mg/L as $CaCO_3$, respectively. Water quality parameters for sites below the ponds in each branch of Trace Fork were not significantly different from one another. There were limited significant differences in water quality in the Lavender Fork watershed. LF-D1 did have significantly higher water hardness compared to LF2 and LF3 with values of 2384 ± 678 mg/L as $CaCO_3$, 1830 ± 526 mg/L as $CaCO_3$, and 1812 ± 554 mg/L as $CaCO_3$. Again, water quality parameters of sites below the pond in Lavender Fork were not significantly different from one another. Furthermore, nearly every site was significantly different ($p < 0.05$) for each water quality parameter when compared to the regional reference site in Five Mile Creek. The pH level for FMC-D was the only parameter that was not significantly different from the regional reference site.

Dissolved water column and total sediment metals were analyzed on one occasion in August 2002 (not reported). The generalized pattern in metal concentrations was that the hollow fill drainages did have higher concentrations compared to mainstem sites or sites below settling ponds. PdR-D2 in the SFPR had a dissolved aluminum concentration in the water column of 787 µg/L (ppb). The highest reported metal concentrations in the SFPR, as well as the entire study, were reported at PdR-AMD, with water column concentrations in ug/L of aluminum, copper, and iron measuring 36.62, 0.028 and 0.742 ug/L. No water column metal measurements in the Powell River watershed were above the U.S EPA's water quality standards (1999), but the metals were higher in PR-D compared to the reference and mainstem sites. Lastly, the West Virginia watersheds had a similar pattern with the fill drainages having higher metal concentrations compared to sites below the settling ponds, although the concentrations were not substantially different.

3.2 Acute Water Column Toxicity Testing

The most toxic site of the study to *C. dubia* was PdR-AMD in the SFPR, which was the area of the upper settling pond drainage influenced by acid mine drainage. Site PdR-AMD had an average LC_{50} of 20.3 ± 4.0% (Table 3a). The second fill drainage in the SFPR was also acutely toxic to *C. dubia* with an average LC_{50} of 60.5 ± 23.2%. The fill drainage in the Powell River watershed had an average LC_{50} of 49.9 ± 13.1%. The only hollow fill to have consistent acute water column toxicity associated with its drainages in West Virginia was in Lavender Fork as fill drainages in LF-D1 and LF-D2 had average LC_{50}s of 71.6 ± 0.8% and 70.6 ± 23.1%, respectively (Table 3b). Additional mortality was recorded in West Virginia at FMC-D and LF3 with 70.7% and 100%; however, this was only on one occasion under low flow conditions.

3.3 Sediment Toxicity Testing

The September sediment toxicity tests for the South Fork of the Pound River and Powell River watersheds did not have any significant *Daphnia* mortality. Sites PdR1 and PR3 had the lowest *Daphnia* survival for the SPFR and Powell River with 33.3 ± 16.9% and 58.3 ± 18.0%, respectively (Table 3a). Sediments from the Powell River watershed did not have any substantial *Daphnia* reproductive impairment. Sediments from the SFPR had varying results for the watershed, with PdR-D2, the second hollow fill drainage, having the highest reproduction with 45.1 ± 8.6. Sites PdR1, PdR2, PdR-D1, and PdR-AMD did have significantly lower reproduction rates compared to PdR-D2.

Sediments collected from West Virginia were analyzed collectively due to similar test conditions in the laboratory. Survival ranged from 100 ± 0 at numerous sites, including Ref, to 33.3 ± 9.5 at both FMC2 and LF-D2 (Table 3b). *Daphnia* reproduction was highest at the Trace Fork fill drainage site, TF-D2, with 62.3 ± 5.0. Overall, FMC1, FMC2, and LF-D2 had significantly lower ($p < 0.05$) reproduction rates than TF-D2 with values of 23.6 ± 8.8, 18.0 ± 17.4, and 16.8 ± 18.6, respectively.

3.4 Benthic Macroinvertebrate Surveys

The SFPR mainstem sites had the highest total richness and EPT richness with 9.3 ± 1.0 and 2.8 ± 0.5 at PdR1 and PdR2, respectively (Table 4a). The lowest total and EPT richness values were reported in the second fill drainage, PdR-D2 with 3.3 ± 0.5, and in the acid mine drainage influenced portion of the tributary, PdR-AMD, with 0.0 ± 0.0. No ephemeopteran taxa (mayflies) were collected at any sampling site in the SFPR. Additionally, it should be noted that the %*Chironomidae* populations were well distributed in the watershed. For instance, the sites with the lowest %*Chironomidae* values were the settling pond drainages, and mainstem sites in the SFPR, which were not significantly different from the percentage in the hollow fill drainages.

The reference site in the Powell River watershed, PRRF, had the overall highest total and EPT richness with 20.8 ± 1.5 and 10.5 ± 1.0, respectively (Table 4a). The lowest total and EPT richness values in the Powell River watershed were at PR-D, the fill drainage, with 3.0 ± 0.8 and 0.5 ± 1.0, respectively. Percent *Ephemeroptera*, a measure of some of the most sensitive organisms in the benthic macroinvertebrate community, was highest at PRRF with the lowest values at PR-D and PR-Da, being 8.9 ± 4.1%, 0.0 ± 0.0%, and 0.0 ± 0.0%, respectively. In contrast, %*Chironomidae* was lowest at PRRF (11.8 ± 5.6%) and the highest values were at PR-D and PR-Da (69.3 ± 16.0%, and 66.0 ± 5.4%, respectively). Percent *Chironomidae* populations in the fill and settling pond drainages were significantly higher ($p < 0.05$) than all other sites in the Powell River watersheds.

Benthic macroinvertebrates collected in West Virginia were compared between sites of the same watershed, with overall comparisons made to the regional reference. First, Five Mile Creek sites had the most significant differences between the fill drainage and sites below the settling ponds. For instance, total and EPT richness were significantly lower in FMC-D, with 11.8 ± 2.2 and 2.0 ± 0.8, respectively, compared to sites below the settling pond (Table 4b). Additionally, FMC-D had lower %Ephemeroptera and elevated %Chironomidae compared to sites below the settling pond, with varying levels of significance. Trace Fork sampling sites, which encompassed two different branches, had limiting significant differences for the watershed. The highest total and EPT richness occurred at TFB, influenced by the older hollow fill, with 17.0 ± 1.4 and 4.0 ± 1.4, respectively. TF1 did have significantly higher ($p < 0.05$) %EPT (31.7 ± 63%) compared to TF-D1 (5.8 ± 9.1%). The %Ephemeroptera and %Chironomidae populations are important to report for Trace Fork. For example, ephemeopterans were almost entirely absent from the watershed, whereas the %Chironomidae populations, which are more tolerant, were evenly distributed. Lastly, for the Lavender Fork watershed, LF-D2 had the lowest total and EPT richness with 9.0

± 1.8 and 2.5 ± 1.0, respectively. The highest total and EPT richness occurred at the most distant downstream site, LF3, with 14.8 ± 1.7 and 6.0 ± 1.4, respectively. The only difference in %EPT occurred at LF-D2 which was significantly lower ($p < 0.05$) than LF3, with 8.5 ± 3.2% and 35.9 ± 4.7%, respectively. Ephemeroptera taxa were absent from the watershed, with %Chironomidae populations evenly distributed.

An interesting pattern concerning benthic macroinvertebrate functional feeding groups was evident in the collector-filterer populations of the studied watersheds of Virginia and West Virginia. Water samples were collected for chlorophyll *a* analysis of the phytoplankton community (not reported) as an estimate of the algal community density in the streams and settling ponds. There was no significant difference in chlorophyll *a* concentrations between the fill drainages and mainstem or sites below the settling ponds. However, the settling ponds did have elevated chlorophyll *a* levels compared to stream sites, which is expected due to the higher concentration of algal communities in these settling ponds. Collector-filterer populations in streams directly below settling ponds were significantly higher than sites directly downstream. The population composition of collector-filterers decreased with increased distance from the settling ponds. The best explanation for the high abundance of collector-filterers at these locations is the high organic enrichment originating from the settling ponds.

3.5 Habitat Assessment

The poorest habitat conditions in the SFPR and Powell River watersheds were located in the fill drainages (Table 2a). The two drainages in the SFPR watershed, PdR-D1 and PdR-D2, scored 95.5 and 111 out of a possible 200 score, respectively. The best habitat conditions in the SFPR were located at PdR3, with 154.5, followed by PdR1 and PdR4, with equal scores of 151.5. The hollow fill drainage in the Powell River, PR-D, had a habitat score of 106.5 whereas PR1 had the highest score of 161.5.

In West Virginia, the average habitat assessment score for each site was very similar to other sites within the same watershed (Table 2b). The hollow fill drainages above the settling ponds had scores ranging from 118 to 144, at LF-D2 and TF-D1, respectively. The major parameters that accounted for the low scores were limited riparian vegetation and poor streambed substrate for the hollow fill drainages. The habitat scores for the sites downstream of the ponds ranged from 107 to 157.5 at TF3 and LF3, respectively. Low habitat scores for sites below settling ponds was attributed to erosion and road influences.

3.6 *In situ* Asian Clam Testing

Asian clams were transplanted to sampling sites for a 60-day period, measuring survival and growth. There was significant ($p < 0.05$) clam mortality in the SFPR at PdR-AMD and PdR-D2 with survival rates of 0.0 ± 0.0% and 20 ± 5.8%, respectively (Table 3a). The remaining sites in the SPFR had clam survival rates ranging from 95 to 100%. Clam growth in the SFPR was highest at PdR-D1a, the upper settling pond drainage, with 2.42 ± 0.58 mm. The SFPR mainstem sites had average clam growths ranging from 0.08 ± 0.03 mm to 0.16 ± 0.03 mm at PdR3 and PdR2, respectively. There was no significant mortality in the Powell River watershed, with the highest overall clam growth occurring at PR-Da, the settling pond drainage, with 0.71 ± 0.05 mm. PR-Da was significantly higher ($p < 0.05$) than all other sites studied in the Powell River watershed. The remaining sites had clam growth rates ranging from 0.01 ± 0.02 mm to 0.16 ± 0.01 mm at PR-D and PRRF, respectively. The fill drainage site, PR-D, had significantly lower clam growth than all sites except for PR2, averaging 0.08 ± 0.03 mm.

The only significant Asian clam mortality in the West Virginia sampling sites occurred in Lavender Fork (Boone County) at LF1 with $65 \pm 10\%$ alive (Table 3b). The growth rates of the Asian clams had a significant pattern across all three watersheds. There was limited growth in the reference site, Ref, as well as the fill drainages with measurements ranging from 0.06 ± 0.03 mm to 0.28 ± 0.03 at Ref and TF-D1, respectively. Limited nutrients and lack of fine particulate organic matter was the logic behind the low growth rates in these areas. The significant clam growth pattern was evident in sites below the settling ponds associated with the fill drainages. In Five Mile Creek and Lavender Fork, growth rates were higher closer to the settling pond compared to the most distant sites. For instance, in Five Mile Creek, FMC1 was significantly higher than FMC2, which was significantly higher than FMC3, with values of 2.42 ± 0.18 mm, 1.68 ± 0.17 mm, and 0.46 ± 0.14 mm, respectively. The pattern was present, but not significant in Lavender Fork. In Trace Fork, TFA, which was below the older settling pond, did have the highest clam growth (2.01 ± 0.06 mm). TFA was significantly higher than TFB, in their respective branch of Trace Fork; however, the clam growth pattern in Five Mile Creek and Lavender Fork was not well established in the other branch of Trace Fork influenced by the younger hollow fill. Overall, it should be noted that the lowest average clam growth occurred in the Ref site.

4. Discussion

Numerous studies pertaining to the influences of coal mining on aquatic systems have focused upon active mining conditions and severe perturbation, such as acid mine drainage and acidification (Guerold et al. 2000; DeNicola et al. 2002; Schmidt et al. 2002). Yet, results of this study did not indicate severe impairment to the water quality or benthic macroinvertebrate communities within the studied stream systems. Elevated conductivity was a common factor among all the sites influenced by hollow fill drainages. These sites were significantly higher than reference sites established in the Powell River watershed in Virginia and Five Mile Creek in West Virginia. Similar research has reported elevated conductivity in being commonly associated with mining influences and drainages and can be used as an indicator of mining activity (García-Criado et al. 1999; Kennedy et al. 2003). The lowest pH of all the study sites discussed was PdR-AMD in the South Fork of the Pound River, which was not directly connected to the hollow fill study. This site was added on to monitor conditions at the acid mine drainage seep and its influence upon the SFPR. Water hardness, which was closely related to conductivity, was elevated at sites influenced by fill drainages, which is an important factor considering metal concentrations within the systems.

Generally, metal concentrations were found to be higher in hollow fill drainages compared to mainstem sites or sites below settling ponds. Water column aluminum, copper, and iron were metals that exceeded the U.S. EPA's WQC (1999) in Virginia and West Virginia. However, water column metal concentrations were lower below the settling ponds, suggesting the ponds were effective buffer zones. The site having the highest metal concentrations in the study was PdR-AMD in the SFPR, which was influenced by a nearby acid mine drainage seep. Again, the conditions of this particular site were not connected to the presence of the hollow fill.

Hollow fill drainages caused minimal toxicity to *Ceriodaphnia* from the water column toxicity testing. Five sites, four hollow fill drainages (PdR-D2, PR-D, LF-D1, LF-D2) and the acid mine drainage influenced tributary (PdR-AMD), were consistentently toxic to this daphnid. The hollow fill drainages did have elevated conductivity and metal concentrations compared to mainstem sites but no acute toxicity. However, the high water hardness in all the systems would suggest metal concentrations would not be the factor contributing to the toxicity because the bioavailable metals would precipitate out. The most toxic site in the entire study was PdR-AMD, which had an average pH of 4.43. Again, this particular site was not directly linked to a hollow fill but had negative attributes originating from a nearby AMD seep.

Sediment collected from the study sites did not provide sufficient information to conclude that sediments from fill drainages were toxic to the test cladoceran, *Daphnia magna*. For instance, in the SFPR, the highest *Daphnia* mortality occurred in PdR1, which was above any potential influence associated with the study. In fact, the daphnid survival rate in the AMD influenced tributary was notably better than PdR1. Additionally, the Powell River watershed and West Virginia watersheds did not have any significant *Daphnia* mortality or reproductive impairment to support the contention that fill drainages were impacted.

Unlike the toxicological data, the benthic macroinvertebrate community analysis provided more insight into the conditions of streams influenced by hollow fill drainages and associated settling ponds. Total richness and EPT richness were lower with varying significance in fill drainages compared to the mainstem and sites below settling ponds. Previous research assessing mine drainages and influences confirmed that taxa abundance and richness are impaired by the potential influences associated with mining (Clements, 1994; Beltman et al. 1999; Soucek et al. 2000). The best explanation for the low richness in the fill drainages is due to the poor habitat conditions there, such as poor riparian vegetation, limited substrate, and reduced water flow.

Benthic macroinvertebrate data from the Powell River and West Virginia watersheds suggested that the benthic macroinvertebrate communities of the fill drainages were a more tolerant community compared to the mainstem sites. A shift from a sensitive based benthic macroinvertebrate community to a tolerant based one in the presences of stressor(s), such as mine drainages, has been reported by others (Roline, 1998; Leland et al. 1989; Clements, 1994). For instance, *Ephemeroptera* were absent from the fill and settling pond drainage in the Powell River watershed and significantly reduced or entirely absent from the fill drainages and sites below the settling ponds in West Virginia. *Ephemeroptera* are well known for their sensitivity to environmental alterations and influences, such as acidic pH and heavy metal burdens (Hickey and Clements 1998; Courtney and Clements 1998; Malmqvist and Hoffsten 1999). On the contrary, the *Chironomidae* populations were elevated at the sites, thereby suggesting a more tolerant benthic macroinvertebrate community. Overall, conditions in the SFPR would suggest stressor(s) influencing the entire watersheds, not just related to hollow fill influences. For instance, the SFPR had no *Ephemeroptera* present; however, the

Chironomidae populations were well distributed meaning more tolerant organisms could inhabit areas in which the sensitive taxa were limited.

Overall, the benthic macroinvertebrate community was not significantly hindered by the drainages originating from the hollow fills. The settling ponds had an integral role in metal and sediment precipitation minimizing potential negative water quality problems downstream. Yet, the benthic community composition was altered due to the presence of the settling ponds. The ponds were a source of organic enrichment in the form of algal communities, which potentially enhanced collector filterer populations. Essentially, the sites directly downstream of the ponds had relatively high collector filterers compared to reference and sites farther downstream. The population composition alteration is important to note because headwater streams are predominately shredder based communities feeding upon leaf litter and woody debris (Short and Maslin, 1977; Vannote et al. 1980). However, in West Virginia, where the streams originated directly from the ponds, collector filterer populations decreased and shredder populations increased with greater distance from the ponds. The alteration in population composition would suggest the streams were returning to a shredder based community in the absence of organic enrichment.

Transplanted bivalve studies have shown significant results evaluating mine drainages and metal influences upon stream systems within the United States and Canada (Belanger et al. 1990; Grout and Levings, 2001; Soucek et al. 2001). *In situ* clam survival, which has proven to be an effective indicator of AMD influence (Cherry et al 2001; Soucek et al. 2000, 2001; Schmidt et al 2002a, b), was not significant in this study. One site did indeed have total clam mortality; however, the PdR-AMD site was influenced by AMD which was not a direct byproduct of the hollow fill drainage. Mortality at the remaining sites could not be directly linked to metal toxicity, due to the elevated water hardness in the watersheds. The most significant pattern evident in the clam study involved growth rates between the fill drainages and mainstem and sites below the settling ponds. In each watershed, both in Virginia and West Virginia, clam growth in sites below the settling ponds was significantly higher compared to hollow fill drainages above the ponds. In Five Mile Creek, clam growth significantly decreased with increased distance from the pond. A similar growth pattern was evident in Lavender Fork below the settling pond; however, there was no significant trend. The best explanation for the enhanced clam growth would be the potential organic enrichment originating from the settling ponds. Clams are collector-filterers, which feed upon fine particulate organic matter. The high algal communities present in the ponds would best explain the optimal growth that occurred directly below the settling ponds.

In conclusion, the water quality and influences originating from the hollow fills did not significantly hinder the benthic macroinvertebrate populations inhabiting these watersheds. The fill drainages did have reduced taxa richness; however, this factor can be attributed to the poor habitat conditions. Conductivity was elevated in studies influenced by hollow fills compared to reference stations, nonetheless this is commonly associated with mining conditions. Metal concentrations were elevated in the fill drainages compared to mainstem sites and others below settling ponds, yet the ponds were effective buffer zones. The most significant alteration to the benthic macroinvertebrate community was connected to the potential organic enrichment from the settling ponds at the base of the hollow fills which enhanced collector filterer populations and optimal growth for Asian clams. Thus, hollow fill drainages and associated settling ponds did influence benthic macroinvertebrate communities of low order streams in Virginia and West Virginia by potentially enhancing a functional feeding group (collector filterers) which out-competed the commonly dominant headwater shredder feeding group.

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Figure 1. Map of the study area within Virginia and West Virginia, USA. Highlighted regions include Wise County in Virginia and Boone County and Mingo County in West Virginia.

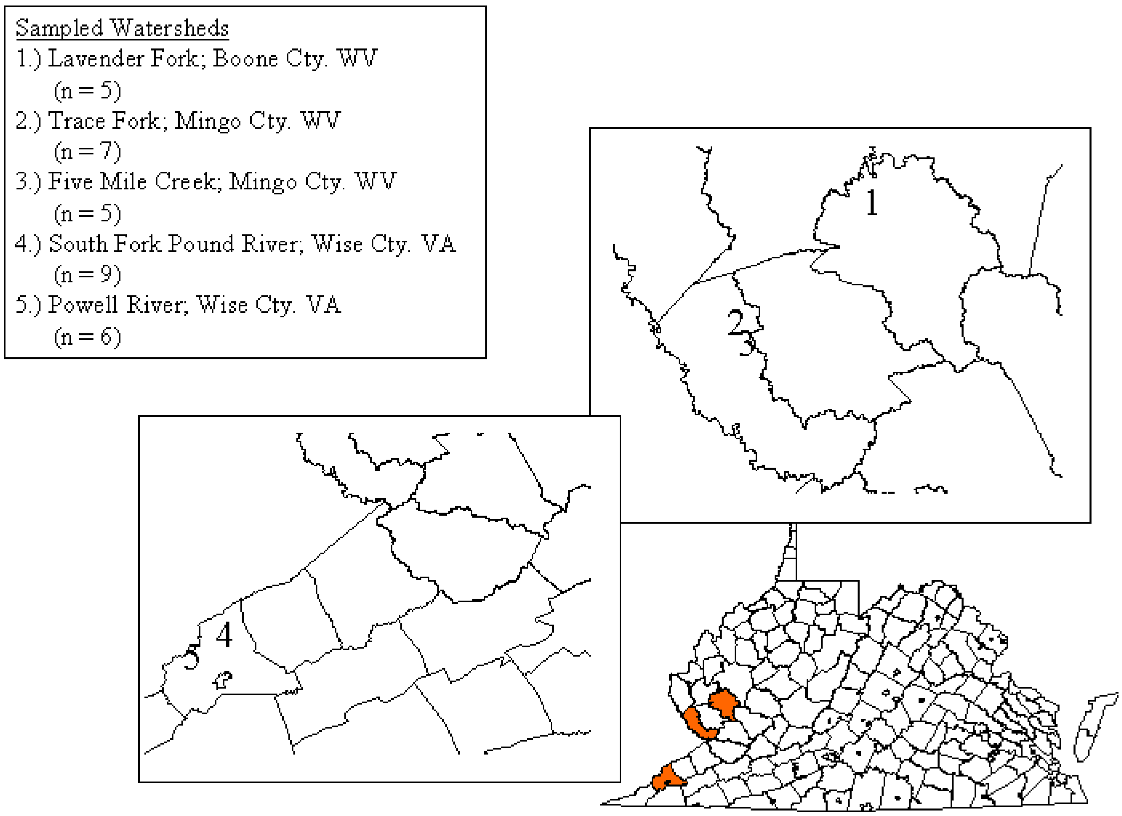

Table 1a. Sampling sites, type classification, and summary of the areas in the South Fork of the Pound River and Powell River watersheds in Wise County, Virginia.

Sites	Type	Description
South Fork Pound River		
PdR-1	Mainstem	Site above drainage from fill and pond of 12 yr. old fill
PdR-D1	Fill Drainage	Drainage originating from a 12 yr. old hollow fill
PdR-D1a	Pond Drainage	Drainage from the settling pond below the 12 yr. old fill
PdR-AMD	AMD	AMD influenced area below the pond drainage tributary
PdR-2	Mainstem	Site below drainage from fill and pond of 12 yr. old fill
PdR-3	Mainstem	Site above drainage from fill and pond of 11 yr. old fill
PdR-D2	Fill Drainage	Drainage originating from a 11 yr. old hollow fill
PdR-D2a	Pond Drainage	Drainage from the settling pond below the 11 yr. old fill
PdR-4	Mainstem	Site below drainage from fill and pond of 11 yr. old fill
Powell River		
PRRF	Reference	Reference area above any mining activity related to fills
PR1	Mainstem	Site adjacent to a 16 yr. old hollow fill
PR2	Mainstem	Site downstream from the 16 yr. old hollow fill
PR-D	Fill Drainage	Drainage originating from a 7 yr. old hollow fill
PR-Da	Pond Drainage	Drainage from the settling pond below the 7 yr. old fill
PR3	Mainstem	Site downstream from the 7 yr. old fill

Table 1b. Sampling sites, type classification, and summary of the areas in Five Mile Creek (Mingo Cty.), Trace Fork (Mingo Cty.), and Lavender Fork (Boone Cty.) in West Virginia.

Sites	Type	Description
Reference Area		
Ref	Reference	Regional reference tributary in Five Mile Creek
Five Mile Creek Sites		
FMC-D	Fill Drainage	Drainage from a 7-yr. old fill
FMC 1, 2, 3	Below Ponds	Sampling sites below the settling pond in 100-150 m Increments
Trace Fork Sites		
TF-D1	Fill Drainage	Drainage from a 3-yr. old fill
TF 1, 2, 3	Below Ponds	Sampling sites below the settling pond in 100-150 m Increments
TF-D2	Fill Drainage	Drainage from a 15-yr. old fill
TF A, B	Below Ponds	Sampling sites below the settling pond in 100-150 m Increments
Lavender Fork Sites		
LF-D1	Fill Drainages	Drainage from a 6-yr. old fill
LF-D2	Fill Drainages	Drainage from a 6-yr. old fill
LF 1, 2, 3	Below Ponds	Sampling sites below the settling pond in 100-150 m Increments

Table 2a. Below is a summary of the water quality parameters measured in the South Fork Pound and Powell River systems in southwestern VA. Habitat assessment (HAS) conditions were measured at each site in June 2002. Mean values and standard deviations are reported.

Sites	Conductivity		pH		Alkalinity		Hardness		HAS
	μS/cm				mg/L as $CaCO_3$		Mg/L as $CaCO_3$		
South Fork Pound River									
PdR1	1632 ± 194	C	8.33 ± 0.3	A	206 ± 26	A	933 ± 98	C	151.5
PdR-D1	2872 ± 173	A	7.81 ± 0.1	BC	103 ± 33	CD	2023 ± 117	A	95.5
PdR-D1a	2716 ± 195	A	7.76 ± 0.2	C	111 ± 57	BCD	2017 ± 281	A	104
PdR-AMD	2654 ± 335	AB	4.43 ± 0.4	D	18 ± 4	E	1113 ± 108	BC	134
PdR2	1657 ± 181	C	8.16 ± 0.3	AB	203 ± 30	A	926 ± 66	C	141
PdR3	1636 ± 214	C	8.15 ± 0.1	A	194 ± 17	AB	919 ± 102	C	154.5
PdR-D2	2245 ± 311	AB	5.78 ± 0.3	D	38 ± 46	DE	1350 ± 121	AB	111
PdR-D2a	2004 ± 192	BC	6.70 ± 0.3	CD	32 ± 4	DE	1297 ± 148	AB	136
PdR4	1631 ± 210	C	8.15 ± 0.1	A	190 ± 19	ABC	936 ± 95	C	151.5
Powell River									
PRRF	944 ± 204	B	7.76 ± 0.3	A	107 ± 20	A	527 ± 124	B	154.5
PR1	941 ± 205	B	7.55 ± 0.1	AB	109 ± 12	A	549 ± 99	B	161.5
PR2	902 ± 175	B	7.61 ± 0.1	A	104 ± 19	A	532 ± 109	B	138.5
PR-D	1748 ± 207	A	7.05 ± 0.4	B	50 ± 9	B	1050 ± 144	A	106.5
PR-Da	985 ± 104	B	7.38 ± 0.1	AB	79 ± 12	AB	537 ± 41	B	142.5
PR3	896 ± 158	B	7.78 ± 0.1	A	104 ± 18	A	493 ± 79	B	139.5

Sites with different letters are significantly different from one another.

Table 2b. Mean water quality data (± standard deviation) for the influence of hollow fill drainages upon headwater streams in WV. Parameters are divided into the reference site, drainages originating from the hollow fills, and sites downstream of the settling ponds associated with the hollow fill.

Water quality and habitat parameters for Five Mile Creek, Trace Fork, and Lavender Fork WV.									
	Conductivity		**pH**		**Alkalinity**		**Hardness**		**Habitat**
Reference Site	µS/cm				mg/L as $CaCO_3$				
Ref	247 ± 87		7.2 ± 0.36		72 ± 52		86± 20		152
Five Mile Creek									
FMC-D	1557 ± 544*	A	7.42 ± 0.20	B	328 ± 88*	A	993 ± 343*	A	132
FMC1	991 ± 421*	B	8.02 ± 0.14*	A	200 ± 70*	B	551 ± 233*	B	138.5
FMC2	965 ± 420*	B	8.37 ± 0.47*	A	196 ± 70*	B	557 ± 232*	B	147
FMC3	923 ± 380*	B	8.14 ± 0.21*	A	216 ± 95*	B	544 ± 226*	B	117.5
Trace Fork									
TF-D1	1310 ± 323*	B	7.84 ± 0.13*	C	240 ± 24*	BC	743 ± 123*	BC	144
TF1	1248 ± 323*	B	8.13 ± 0.03*	A	222 ± 20*	C	699 ± 121*	C	146
TF2	1231 ± 312*	B	8.15 ± 0.03*	A	222 ± 30*	C	710 ± 129*	BC	142.5
TF3	1200 ± 288*	B	8.15 ± 0.08*	A	217 ± 26*	C	687 ± 121*	C	107
TF-D2	1643 ± 370*	A	7.98 ± 0.09*	B	352 ± 35*	A	1078 ± 182*	A	127.5
TFA	1355 ± 352*	AB	8.13 ± 0.06*	A	265 ± 37*	B	855 ± 204*	B	134
TFB	1310 ± 327*	B	8.22 ± 0.06*	A	258 ± 34*	BC	819 ± 196*	BC	119.5
Lavender Fork									
LF-D1	3050 ± 883*	A	8.09 ± 0.03*	AB	239 ± 23*	A	2384 ± 678*	A	126
LF-D2	2497 ± 780*	A	7.93 ± 0.18*	B	296 ± 77*	A	1882 ± 707*	AB	118
LF1	2720 ± 929*	A	8.09 ± 0.05*	AB	217 ± 62*	A	1904 ± 596*	AB	130
LF2	2667 ± 939*	A	8.10 ± 0.06*	AB	218 ± 59*	A	1830 ± 526*	B	140
LF3	2657 ± 956*	A	8.11 ± 0.08*	A	218 ± 63*	A	1812 ± 554*	B	157.5

* Denotes significant differences from the reference site

Sites with different letters are significantly different from one another.

Table 3a. Toxicological data from the South Fork of the Pound River and Powell River watersheds in Virginia.

	WC Testing	Sediment Toxicity Testing				In situ Asian Clam Test			
	2002	Sep-02				2002			
Sites	LC**50** (%)	Reproduction		Survival (%)		60-Day Growth		Survival (%)	
South Fork Pound River									
PdR1	n/a	10.7 ± 13.4	D	33.3 ± 16.9	A	0.14 ± 0.04	CD	95 ± 2.5	A
PdR-D1	n/a	20.7 ± 12.0	BCD	50 ± 20.8	A	0.17 ± 0.04	BC	100 ± 0.0	A
PdR-D1a	n/a	34.3 ± 3.8	ABC	100 ± 0	A	2.42 ± 0.58	A	95 ± 2.5	A
PdR-AMD	20.3 ± 4.0	16.5 ± 6.9	D	91.7 ± 6	A	0.00 ± 0.00	F	0.0 ± 0.0	B
PdR2	N/a	18.3 ± 9.6	CD	66.7 ± 16.9	A	0.16 ± 0.03	C	95 ± 2.5	A
PdR3	N/a	26.5 ± 17.9	ABCD	75 ± 18	A	0.08 ± 0.03	E	95 ± 2.5	A
PdR-D2	60.5 ± 23.2	34.5 ± 1.0	AB	91.7 ± 6	A	0.01 ± 0.01	F	20 ± 5.8	B
PdR-D2a	N/a	45.1 ± 8.6	A	100 ± 0	A	1.39 ± 0.32	AB	100 ± 0.0	A
PdR4	N/a	30.5 ± 2.8	ABCD	91.7 ± 6	A	0.11 ± 0.02	DE	100 ± 0.0	A
Powell River									
PRRF	n/a	32.9 ± 5.0	A	100 ± 0	A	0.16 ± 0.01	B	90 ± 2.9	A
PR1	n/a	22.3 ± 3.8	A	100 ± 0	A	0.11 ± 0.04	C	100 ± 0.0	A
PR2	n/a	25.2 ± 8.4	A	83.3 ± 12	A	0.08 ± 0.03	CD	95 ± 2.5	A
PR-D	49.9 ± 13.1	30.2 ± 0.8	A	100 ± 0	A	0.01 ± 0.02	D	100 ± 0.0	A
PR-Da	n/a	32.1 ± 2.5	A	100 ± 0	A	0.71 ± 0.26	A	100 ± 0.0	A
PR3	n/a	26.2 ± 19.4	A	58.3 ± 18	A	0.12 ± 0.05	BC	90 ± 2.9	A

Sites with different letters are significantly different from one another.

Table 3b. Toxicological data from the Five Mile Creek, Trace Fork, and Lavender Fork watersheds in West Virginia.

Research Sites	*C. dubia* LC50 (%)	*D. magna* Sediment Toxicity Testing Reproduction		Survival (%)		Asian Clam Toxicity Test 60-Day Growth mm		Survival %	
Reference Site									
Ref	n/a	41.4 ± 1.4	AB	100 ± 0.0	A	0.06 ± 0.03		100 ± 0	
<u>Five Mile Creek</u>									
FMC-D	70.71	34.9 ± 13.4	AB	83.3 ± 8.3	AB	0.23 ± 0.04	C	100 ± 0	A
FMC1	n/a	23.6 ± 8.8	B	75 ± 8.0	AB	2.42 ± 0.18	A*	95 ± 10	A
FMC2	n/a	18.0 ± 17.4	B	33.3 ± 9.5	B	1.68 ± 0.17	B*	100 ± 0	A
FMC3	n/a	27.6 ± 18.9	AB	75 ± 12.5	AB	0.46 ± 0.14	C*	100 ± 0	A
<u>Trace Fork</u>									
TF-D1	n/a	41.4 ± 8.0	AB	91.7 ± 4.2	AB	0.28 ± 0.03	D	95 ± 10	A
TF-1	n/a	36.9 ± 11.8	AB	83.3 ± 8.3	AB	1.29 ± 0.16	BC*	100 ± 0	A
TF-2	n/a	40.8 ± 1.8	AB	100 ± 0.0	A	1.01 ± 0.03	C*	85 ± 19	A
TF-3	n/a	37.1 ± 9.0	AB	100 ± 0.0	A	1.46 ± 0.33	B*	95 ± 10	A
TF-D2	n/a	62.3 ± 5.0	A	100 ± 0.0	A	0.18 ± 0.04	D	100 ± 0	A
TFA	n/a	37.5 ± 4.9	AB	100 ± 0.0	A	2.01 ± 0.06	A*	95 ± 10	A
TFB	n/a	41.4 ± 4.3	AB	100 ± 0.0	A	1.06 ± 0.16	C*	95 ± 10	A
<u>Lavender Fork</u>									
LF-D1	71.63 ± 0.80	36.9 ± 1.8	AB	93.3 ± 4.2	AB	0.16 ± 0.02	B	100 ± 0	A
LF-D2	70.63 ± 23.1	16.8 ± 18.6	B	33.3 ± 9.5	B	0.22 ± 0.03	B	100 ± 0	A
LF1	n/a	39.5 ± 2.7	AB	100 ± 0.0	A	0.77 ± 0.09	A*	65 ± 10	B*
LF2	n/a	30.5 ± 20.8	AB	75 ± 12.5	AB	0.34 ± 0.11	A	85 ± 19	AB
LF3	100	35.1 ± 7.9	AB	100 ± 0.0	A	0.17 ± 0.14	A	95 ± 10	A

* Sites with different letters are significantly different from one another.

Table 4a. Benthic macroinvertebrate community data for the South Fork of the Pound River and Powell River watersheds in Virginia.

Benthic Macroinvertebrate Indices for South Fork Pound River and Powell River, VA, USA.										
South Fork Pound River										
Sites	Richness		EPT Richness		% EPT		% Ephemeroptera		%Chironomidae	
PdR1	9.3 ± 1.0	A	2.5 ± 0.6	AB	24.6 ± 10.1	B	0.0 ± 0.0	A	44.6 ± 17.3	AB
PdR-D1	5.0 ± 1.8	BCD	0.3 ± 0.5	DE	2.3 ± 4.6	D	0.0 ± 0.0	A	44.9 ± 17.8	AB
PdR-D1a	7.0 ± 1.8	ABC	1.0 ± 0.0	CD	71.9 ± 3.1	A	0.0 ± 0.0	A	10.6 ± 3.5	C
PdR-AMD	4.3 ± 1.5	CD	0.0 ± 0.0	E	0.0 ± 0.0	D	0.0 ± 0.0	A	30.5 ± 3.4	BC
PdR2	7.8 ± 1.7	AB	2.8 ± 0.5	A	27.3 ± 1.3	BC	0.0 ± 0.0	A	51.7 ± 6.6	AB
PdR3	7.3 ± 1.0	ABC	2.5 ± 0.6	AB	34.9 ± 5.9	AB	0.0 ± 0.0	A	34.7 ± 6.3	ABC
PdR-D2	3.3 ± 0.5	D	0.3 ± 0.5	DE	5.0 ± 10.0	CD	0.0 ± 0.0	A	57.5 ± 5.0	A
PdR-D2a	3.8 ± 1.0	D	1.3 ± 0.5	BCD	30.8 ± 27.6	BC	0.0 ± 0.0	A	26.3 ± 17.1	BC
PdR4	4.3 ± 1.3	CD	1.5 ± 1.0	ABC	44.3 ± 17.7	AB	0.0 ± 0.0	A	37.1 ± 10.1	ABC
Powell River										
PRRF	20.8 ± 1.5	A	10.5 ± 1.0	A	38.2 ± 11.5	A	8.9 ± 4.1	A	11.8 ± 5.6	B
PR1	10.3 ± 4.4	B	3.8 ± 2.1	BC	31.9 ± 12.8	AB	7.6 ± 11.2	AB	22.8 ± 8.8	B
PR2	9.3 ± 2.1	B	4.0 ± 2.3	B	35.7 ± 21.6	AB	8.7 ± 3.6	A	25.1 ± 8.0	B
PR-D	3.0 ± 0.8	C	0.5 ± 1.0	C	7.1 ± 14.3	B	0.0 ± 0.0	B	69.3 ± 16.0	A
PR-Da	7.3 ± 1.7	BC	1.8 ± 1.0	BC	9.8 ± 3.7	AB	0.0 ± 0.0	B	66.0 ± 5.4	A
PR3	11.0 ± 1.4	B	2.8 ± 0.5	BC	18.3 ± 6.5	AB	5.4 ± 3.8	AB	27.2 ± 10.3	B

Sites with different letters are significantly different from one another.

Table 4b. Benthic macroinvertebrate community data for the Five Mile Creek, Trace Fork, and Lavender Fork watersheds in West Virginia.

Benthic macroinvertebrate data for research stations influenced by hollow fill drainages in southern West Virginia										
Stations	**Total Richness**		**EPT Richness**		**%EPT**		**%Ephemeroptera**		**%Chironomidae**	
Reference Station										
Ref	15.8 ± 2.5		7.5 ± 1.0		73.4 ± 3.4		16.9 ± 7.7		9.8 ± 3.3	
Five Mile Creek										
FMC-D	11.8 ± 2.2	B	2.0 ± 0.8	C*	5.8 ± 3.0	B*	0.63 ± 1.2	B*	53.3 ± 7.5	A*
FMC1	19.5 ± 2.1	A	6.8 ± 1.5	B	17.4 ± 3.1	AB*	6.1 ± 2.0	A*	35.6 ± 9.6	AB
FMC2	21.5 ± 3.3	A*	9.5 ± 1.3	A	18.3 ± 4.0	AB*	2.5 ± 1.6	B*	28.5 ± 14.2	B
FMC3	18.8 ± 1.0	A	7.3 ± 1.0	AB	28.5 ± 16.5	A*	2.7 ± 1.5	B*	24.3 ± 6.1	B
Trace Fork										
TF-D1	10.0 ± 2.9	B*	1.0 ± 1.4	B*	5.8 ± 9.1	B*	0	A*	32.0 ± 15.1	A
TF-1	10.3 ± 2.2	B	2.8 ± 1.5	AB*	31.7 ± 6.3	A*	2.0 ± 1.7	A*	18.5 ± 1.2	A
TF-2	11.3 ± 1.3	B	2.0 ± 0.8	AB*	18.1 ± 1.0	AB*	0	A*	19.5 ± 4.6	A
TF-3	10.8 ± 2.2	B	2.0 ± 0.8	AB*	15.2 ± 1.4	AB*	1.7 ± 2.0	A*	35.6 ± 6.4	A
TF-D2	10.0 ± 2.7	B*	1.8 ± 1.7	AB*	19.3 ± 22.2	AB*	0	A*	41.5 ± 19.6	A*
TFA	13.3 ± 1.5	AB	3.3 ± 1.0	AB*	19.5 ± 6.6	AB*	0	A*	29.7 ± 12.1	A
TFB	17.0 ± 1.4	A	4.0 ± 1.4	A*	23.1 ± 8.8	AB*	0	A*	33.5 ± 7.9	A
Lavender Fork										
LF-D1	10.5 ± 2.4	AB	3.0 ± 0.8	B*	8.5 ± 3.2	B*	0	A*	49.3 ± 11.2	A*
LF-D2	9.0 ± 1.8	B*	2.5 ± 1.0	B*	23.8 ± 18.0	AB*	0	A*	30.8 ± 14.8	A
LF1	12.3 ± 2.4	AB	4.8 ± 1.0	AB	32.3 ± 8.3	AB*	0	A*	35.6 ± 7.7	A
LF2	12.5 ± 1.7	AB	5.5 ± 1.3	A	32.3 ± 15.9	AB*	0	A*	53.2 ± 14.9	A*
LF3	14.8 ± 1.7	A	6.0 ± 1.4	A	35.9 ± 4.7	A*	0	A*	37.2 ± 9.4	A

* Denotes significant differences from the reference site.

Sites with different letters are significantly different from one another.