Ecotoxicological Evaluation of Hollow Fill Drainages in Low Order Streams in the Appalachian Mountains of Virginia and West Virginia

by

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Abstract

Hollow fills are composed of excess spoil and debris produced from surface coal mining that is not returned to the original mined site. Hollow fills are often constructed in the head of hollows nearby or adjacent to the mined land area, which may be the origins of headwater streams or drain into low order systems. Eleven hollow fills were utilized in evaluating the influence fill drainages had on low order streams in Virginia and West Virginia. The study was conducted in six watersheds including; Five Mile Creek in Mingo County, West Virginia, Trace Fork in Mingo County, West Virginia, Lavender Fork in Boone County, West Virginia, Middle Creek in Tazewell County, Virginia, South Fork of the Pound River in Wise County, Virginia, and Powell River in Wise County, Virginia. Bioassessment procedures used in the evaluation of hollow fill drainages included water/sediment chemistry, acute water column toxicity testing using *Ceriodaphnia dubia*, chronic sediment toxicity testing using *Daphnia magna*, benthic macroinvertebrate surveys, and *in situ* Asian clam (*Corbicula fluminea* [Müller]) toxicity testing. Common significant differences in water quality between reference and fill influenced sites, among all watersheds, were elevated conductivity and water column metal concentrations, particularly aluminum and copper. Water column and sediment toxicity testing reported limited significant mortality or reproductive impairment associated with hollow fill drainages. The West Virginia watersheds used in the study

consisted of headwater streams originating directly from the settling ponds, placed at the base of the hollow fills, receiving drainages from the fills. Benthic macroinvertebrate analysis reported no significant alteration in total taxa or EPT richness downstream of the ponds. Yet, collector filterer populations, including benthic macroinvertebrates and *in situ* Asian clams, were enhanced directly downstream of the ponds due to organic enrichment originating from the ponds. A decrease in collector filterer populations and lowered clam growth suggested the organic enrichment dissipated downstream from the ponds. Chlorophyll *a* analysis of the phytoplankton community was not significantly related to the enhance collector filterer populations in the streams, however the high concentrations in the settling ponds suggest abundant algal communities. The hollow fills evaluated in Virginia drained into receiving systems, whose headwater origins were not directly related to hollow fill drainages. Low taxa richness was associated with the hollow fill and settling pond drainages, however receiving system sites were minimally influenced. Yet, as reported in the West Virginia watersheds, the settling ponds input organic enrichment that enhanced collector filterer populations, including benthic macroinvertebrates and *in situ* test clams. An analysis of the hollow fills' age, or maturity, reported no significant difference between young and old fills. In general, a common feature of among the various aged fill drainages was elevated conductivity, compared to reference sites of the watersheds.

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Introduction

Studies have been conducted on numerous disturbances to streams in the Appalachian Mountains ranging from mined land reclamation (Matter and Ney, 1981), acid mine drainage (Cherry et al. 2001; Soucek et al. 2001), to industrial discharges (Farris et al. 1998). Surface coal mining is a major industry within the Appalachia region, with activities negatively influencing both terrestrial and aquatic habitats. Legislation, including the Clean Water Act (1977) and Surface Mining Control and Reclamation Act of 1977, regulates and permits mining activities to minimize the negative impact of the operations upon the environment. The influences associated with active mining have been well researched, however the longterm implication of hollow fill drainages upon low order streams is not well studied.

Hollow fills are constructed from excess spoil and debris, or overburden, produced from surface coal mining that is not returned to the original mined land area. The overburden is normally disposed of in nearby or adjacent hollows, which may be the origin of headwater streams, or may drain into low order streams, as in the Appalachian region. A method of passive control for the hollow fill drainages is the placement of settling ponds at the toe or base of the fills to impede the drainages from flowing directly into the receiving streams. The presence of the settling ponds, impeding the fill drainages before discharging into the streams, was essential in sediment deposition and potential metal dilution and precipitation.

Understanding the influences and implications of mine drainages is important to the ensuring water standards are upheld and the issue of future surface mining permits. Numerous researchers have evaluated types of influences that may be associated with

mine drainages including; stream sedimentation and loss of habitat (Bonta, 2000), stream acidification (Courtney and Clements, 1998), elevated conductivity (García-Criado et al. 1999; Kennedy et al. 2003), metal influx and precipitation (Roline, 1988; Clements, 1994; Milan and Farris, 1998; Beltman et al. 1999), as well as acid mine drainage (Kiffney and Clements, 1993; Cherry et al. 2000; Soucek et al. 2001). Research on negative factors associated with mine drainages has reported benthic macroinvertebrate communities impacted with a decrease of taxa richness and loss of sensitive taxa (Roline, 1998; Leland et al. 1989). Studies have stated the recovery of streams after severe perturbations, such as mine drainages, if the hindering factor(s), such as metal contamination, are eliminated (Nelson and Roline, 1996; Jeffree et al. 2001; DeNicola and Stapleton, 2002).

The major objective of this research was to evaluate the influences hollow fill drainages had upon water quality and benthic macroinvertebrate communities in low order streams in Virginia and West Virginia. The study focused on eleven hollow fills in six different watersheds including Lavender Fork, Trace Fork, and Five Mile Creek in West Virginia as well as Middle Creek, South Fork of the Pound River, and Powell River in Virginia. Bioassessment procedures used in the evaluation included water/sediment chemistry, acute water column toxicity testing with *Ceriodaphnia dubia*, chronic sediment toxicity testing with *Daphnia magna*, benthic macroinvertebrate surveys, and *in situ* Asian clam (*Corbicula fluminea*) toxicity testing.

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1 Alterations in West Virginia headwater streams due to head of hollow fill and associated settling pond drainages.

Abstract. The attributes and long-term influences associated with coal mining spoil generated hollow fills and associated settling ponds were assessed in three headwater streams in southern West Virginia. Drainages originating from the hollow fills, above the settling ponds, reported elevated conductivity and metal concentrations, particularly aluminum and copper, compared to a regional reference station. Benthic macroinvertebrate sampling and *in situ* Asian clam testing were utilized in assessing the hollow fills' influences upon these low order streams. All research stations with an average conductivity of approximately 1500 μS/cm or above lacked *Ephemeroptera* taxa. Overall benthic macroinvertebrate richness was not severely hindered by the hollow fill drainages. The most significant alteration to the macroinvertebrate community appeared to be the potential organic enrichment from the settling ponds that augmented collector filterer populations. The benthic macroinvertebrates communities downstream of the settling ponds ranged from 26%-50% collector filterers, compared to the 1% collector filterers and 50% shredder based community in the reference station. Asian clam growth was enhanced by the presence of the settling ponds, with the greatest 60-day growth occurring directly downstream of the ponds, with growth deceasing with distance from the ponds. An application of an ETR (ecotoxicological rating) procedure for all watersheds in the study suggested all hollow fill influenced stations were environmentally stressed, compared to the reference.

1.1 Introduction

Bioassessment procedures have been utilized in evaluating aquatic systems impacted by various disturbances such as land usage (Williams et al. 2002), industrial discharges (Diamond et al. 2002), and mine drainages (Soucek et al. 2000; Cherry et al.

2001; DeNicola and Stapleton, 2002). Research has been conducted on different types of environmental impacts associated with coal mining ranging from stream acidification (Guerold et al. 2000), sedimentation of stream habitat (Bonta, 2000), heavy metal influx (Clements et al. 2000), and acid mine drainage (Cherry et al. 2001; Soucek et al. 2001a). Impacts of this nature have detrimental influences on the chemical composition and biota of aquatic systems potentially resulting in loss of sensitive species, such as bivalves and *Ephemeroptera* (Leland et al. 1989; Clements, 1994; Guerold et al. 2000). Significant research has been conducted on impacts associated with active coal mining and acid mine drainage, yet interest has increased concerning the influences of hollow fill drainages upon headwater and low order streams.

Headwater streams are ecologically significant locations in aquatic systems having intimate relationships with their riparian surroundings and receiving nutrient input in the form of leaf litter and woody debris. Normally, headwater streams have high abundances of organisms feeding upon the coarse particulate organic matter (CPOM), including benthic macroinvertebrate shredders. These shredder communities feed on and process the CPOM into fine particulate organic matter (FPOM), which is a food source for communities downstream including collector filterers and gatherers (Short & Maslin 1977; Vannote et al. 1980). The typical disturbances to headwaters streams include mining activities and clear-cut logging, which influence the terrestrial environment as well as the aquatic. Yet, industrial actions, such as mining, are permitted under federal and state laws to ensure the environment is properly protected.

In 1977, laws controlling environmental impacts associated with coal mining were established, including the Surface Mining Control and Reclamation Act (SMCRA)

and amendments to the 1972 Federal Water Pollution Control Act to create the 1977 Clean Water Act (CWA). Active mining facilities were required to obtain permits for point source discharges entering into any U.S. surface waterway and were regulated under those permits to ensure water quality standards were upheld. The SMCRA and the CWA, along with other forms of legislation, ensure minimal impact upon the environment and proper reclamation of mined lands upon completion of mining activities.

Head of hollow fills are a surface mining byproduct, produced from excess soil and debris or overburden, that is not returned to the original mine site. The excess inter- and overburden soil and debris are removed to gain access the underlying coal seam resulting in material for hollow fill construction in nearby or adjacent hollows (Fung, 1981). Settling ponds are normally placed at the toe of the hollow fill for sediment and metal precipitation that prove to be beneficial buffer zones (Chadwick and Canton, 1983). The hollows in which these fills are constructed may be the origins of headwater streams, or drainages and seeps from the fills may enter and influence low order systems.

The objective of this study concerned the effects hollow fill drainages had on the abiotic and biotic conditions within headwater streams in southern West Virginia. 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]). Interest laid in the physiochemical influences originating from the hollow fills and their subsequent effects upon water quality and benthic macroinvertebrates of the headwater streams. The headwater streams evaluated in the

study originated directly from the fill drainages and associated settling ponds. The settling ponds were of particular interest since their presence potentially provided organic enrichment to the headwater stream that would not normally be present.

1.2 Methods

1.2.1 Sampling Stations

Sampling occurred seasonally in 2002 in the Appalachia region of southern West Virginia (Figure 1.1). Seventeen sampling stations were assessed and surveyed in three different watersheds evaluating four hollow fill drainages and associated settling ponds. The stations were sampled to evaluate fill drainages above the settling ponds at the base of the fills and in multiple locations downstream of the ponds. The drainages originating from the hollow fills were constantly flowing throughout the sampling seasons. Thus, five stations were established in hollow fill drainages, eleven stations were established downstream of the settling ponds, and a regional reference station above any mining influence was established in one of the watersheds. Site descriptions are summarized in Table 1.1.

Five sampling stations were established within the Five Mile Creek watershed in Mingo County, West Virginia. The hollow fill was completed seven years ago (1995) and has a volume of 122,100 cubic yards with grass as the dominant terrestrial vegetation including scattered shrubs and small trees. One station was established in the fill drainage above the settling pond, with three stations placed downstream of the pond in 100-150 yd. increments. Additionally, a small tributary above any hollow fill or mining influence was established in the Five Mile Creek watershed as a regional reference station.

Seven sampling stations 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 three year old hollow fill (1999) with a volume of 317,504 cubic yards and limited vegetation, consisting of mainly grass. One station was established in the fill drainage above the settling pond with three stations established downstream of the pond in 100-150 yds. increments. A second branch of Trace Fork was influenced by a fifteen year old hollow fill completed in (1987). The older hollow fill has a volume of 64,312 cubic yards with well established grass, tree, and shrub vegetation. One research station was established in the fill drainage originating from the toe of the fill above the settling pond with two stations established downstream of the pond in 100-150 yds. increments.

Five sampling stations were established in the Lavender Fork watershed in Boone County, West Virginia. The hollow fill utilized in the study was six years old (1996) with a volume of 115,840 cubic yards with grass as the dominant vegetation including established shrubs and small trees. One station was established in each of the two drainages originating from the fill above the settling pond, with three stations established downstream of the pond in 100-150 yds. increments.

1.2.2 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. 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.

1.2.3 Chlorophyll *a* Analysis

The chlorophyll *a* concentrations of the phytoplankton communities of the fill drainages, settling ponds, and stations downstream of the ponds were measured to evaluate the algal communities within those areas. Chlorophyll *a* concentrations were measured by collecting 1-L grab samples from each station and pond and filtering the sample (pore size 0.47 μm). The filter paper was ground in a tissue grinder in an acetone solution and clarified by centrifugation. The prepared samples were evaluated using the spectrophotometer method detailed in the Standard Methods for the Examination of Water and Wastewater (APHA 1995).

1.2.4 Water Column Toxicity Testing

Water samples were collected seasonally in 1-L high density polyethylene bottles for toxicity testing upon the test 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 involved a 0.5 serial dilution of the collected station 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).

1.2.5 Sediment Toxicity Testing

Sediment samples were collected at the seventeen stations (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. 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 station. 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. Sediment from the regional reference station in Five Mile Creek was used as a control for the test.

1.2.6 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 station. 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. In addition, the Hilsenhoff Biotic Index (Hilsenhoff, 1987) was utilized in analyzing the tolerance of the taxa occurring at each station. The HBI is a benthic index to assess organic pollution in which the lower value represents low organic pollution and a higher pertains to high organic pollution.

1.2.7 Habitat Assessment

Habitat conditions were assessed at each station 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 station, the better the habitat conditions. Two

individual researchers conducted the habitat assessment surveys separately and mean scores for each station 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.

1.2.8 *In situ* Asian clam toxicity testing

Asian clams (*Corbicula fluminea* [Müller]), collected from reference stations 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 stations. Four replicates (20 clams) were fastened and arranged around a piece of rebar and placed in riffles at each research station. 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.

1.2.9 Ecotoxicological Rating Procedure

An ecotoxicological rating (ETR) procedure was developed to better evaluate the environmental conditions at the sampling stations within the study. The ETR utilized in this study was based on ETRs implemented in southwestern VA in similar research (Cherry et al. 2001; Soucek et al. 2000). Ecological, toxicological, and physiochemical parameters were selected for analysis by each parameter's relevance to mine drainage

influences on aquatic systems. The ecological measurements of total richness, EPT richness, %EPT, %*Ephemeroptera*, and %*Chironomidae* were selected due to the sensitivity or tolerance of the parameter. Transplanted Asian clam (*C. fluminea*) growth was selected as the toxicology measure due to growth sensitivity in the presence of mining toxicants (Grout and Levings, 2001; Soucek et al. 2001a). Also, physicochemical parameters, such as conductivity, dissolved Al and Cu concentrations in the water column, and habitat analyses, were selected due to relevance in similar research (Soucek et al. 2001b; Schmidt et al. 2002: Kennedy et al. 2003).

All parameters, except conductivity, %*Chironomidae*, and dissolved Al and Cu, were transformed into a percentage with the highest value measured equaling 1 and subsequent values were then divided by the highest value (value measured at a site/highest value measured in the watershed). Conductivity and %*Chironomidae* measurements were transformed into a percentage with the lowest value measured equaling 1 and subsequent values were then used to divide the lowest value (lowest value measured in the watershed/value measured at a site). Dissolved Al and Cu concentrations for each site were assessed with values under U.S. EPA water quality criteria (WQC) (1999) receiving a 1.0 and values over criteria receiving a 0.1. The ecological parameters of total richness, EPT richness, % *Ephemeroptera*, and %EPT were multiplied by 12.5 due to their ecological sensitivity. The parameters of %*Chironomidae* and conductivity were multiplied by 10, and dissolved Al and Cu, habitat assessment, and Asian clam growth were multiplied by 7.5. The highest cumulative score a site could obtain was 100. The ETR works on a basis of the higher the total score, the less environmentally impacted

the site. Cherry et al. (2001) developed a percentile ranking whereby sites scoring 90% were excellent, 80% were acceptable, 70% were marginal, and ≤ 60% were stressed.

1.2.10 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®.

1.3 Results

1.3.1 Water Column & Sediment Chemistry

The reference station, Ref, had mean water quality measurements of 247μS/cm, 72 mg/L as $CaCO_3$, and 86 mg/L as $CaCO_3$ for conductivity, alkalinity, and hardness, respectively, which were the overall lowest levels for all stations (Table 1.2). Mean water quality measurements for all hollow fill influenced stations were 1570 μS/cm, 223 mg/L, and 998 mg/L for the same measurements, respectively. The mean conductivity at Ref, 247 ± 87 μS/cm was significantly lower ($p < 0.05$) than FMC-D, TF-D2, LF-D1, LF-D2, LF1, LF2, and LF3 which all had an average conductivity of 2127 ± 975 μS/cm. Significant differences ($p < 0.05$) were found concerning pH between Ref and fill influenced stations, however pH among all stations ranged from 7.20 to 8.37, at Ref and FMC2, respectively. Water hardness was directly related to conductivity ($p < 0.0001$),

with Ref having significantly lower water hardness, 86 ± 20 mg/L as $CaCO_3$ compared to hollow fill drainages and stations in Lavender Fork and Trace Fork. Water hardness in the fill drainages ranged from 743 ± 123 mg/L to 2384 ± 678 mg/L at TF-D1 and LF-D2, respectively. Stations downstream of the settling ponds ranged in water hardness from 544 ± 226 mg/L at FMC3 to 1904 ± 596 mg/L at LF1.

In general, the majority of the fill influenced stations had higher metal concentrations in the water column and sediments compared to the reference, Ref. (Table 1.3). Aluminum concentrations, ranging from 0.113 mg/L to 0.267 mg/L, were above the 1999 U.S. EPA's nationally recommended water quality criteria (0.087 mg/L) at all stations except for the reference and Five Mile Creek stations, FMC1-FMC3. Both copper and iron concentrations were elevated at stations influenced by hollow fill drainages compared to Ref. Water column metal concentrations were lower at stations downstream of the ponds compared fill drainage stations above the ponds. Unlike the water column metals, sediment metal concentrations of the stations downstream of the ponds were not consistently lower than fill drainage stations.

1.3.2 Chlorophyll *a* Analysis

One liter grab samples collected from the hollow fill drainages and stations below the settling ponds did not report distinct differences from one another (Table 1.4). Additionally, there was no apparent pattern or trend represented by the chlorophyll *a* concentrations below the ponds. Yet, the chlorophyll *a* measurements of the phytoplankton communities in the ponds were, in general, elevated well above both the fill drainages and sites below the ponds.

1.3.3 Water Column Toxicity Testing

Water column toxicity was only prominent in the two drainages originating from the hollow fill in Lavender Fork with mean LC_{50}s averaging 71.63 ± 0.80 and 70.63 ± 23.1 for LF-D1 and LF-D2, respectively (Table 1.5). The Lavender Fork fill drainage stations, LF-D1 and LF-D2, were the only stations with *Ceriodaphnia* mortality for every water sample collected. Single occurrence mortality occurred late in the sampling season under low flow conditions, with LC_{50}s of 70.71 and 100 at FMC-D and LF3, respectively, with no mortality evident during the remainder of the sampling season.

1.3.4 Sediment Toxicity Testing

Lavender Fork and Five Mile Creek stations, LF-D2 and FMC2, had significant *Daphnia* mortality ($p < 0.05$) compared to Ref, with only 33.3% of the test cladocerans surviving the chronic exposure (Table 1.5). Overall, *Daphnia* fecundity was significantly reduced ($p < 0.05$) at a Lavender Fork station, LF-D2, and Five Mile Creek stations, FMC1 and FMC2 with mean neonate reproductive values of 16.8 ± 18.6, 23.6 ± 8.8, and 18.0 ± 17.4, respectively. The highest *Daphnia* fecundity among the stations was 62.3 ± 5.0 at the Trace Fork hollow fill drainage, TF-D2. No metal analyzed could be significantly correlated the mortality or reduced fecundity in the chronic test.

1.3.5 Benthic Macroinvertebrate Sampling

Benthic macroinvertebrate data were analyzed in multiple patterns to distinguish any potential influence within the watersheds studied. First, the streams were analyzed separately between the different watersheds in order to eliminate the assumption that each system was subjected to similar influences. Therefore, the hollow fill drainages were evaluated separately to compare all fill drainages and streams originating downstream of

the settling ponds were analyzed separately. Comparisons were made between the regional reference station and all of the stations to evaluate alterations in influenced systems compared to an uninfluenced headwater stream. Additionally, a two factor design was utilized in evaluating the downstream sites for all of the watersheds based upon their proximity to the settling pond. The data concerning the benthic macroinvertebrate community indices are summarized in Table 1.6 and Table 1.7.

Total richness, EPT richness, %EPT, %*Ephemeroptera*, and %*Chironomidae* were not significantly different among all of the fill drainage stations (Table 1.6). Yet, compared to the total richness of 15.8 ± 2.5 at Ref, three hollow fill drainages, TF-D1, TF-D2, and LF-D2, had significantly lower ($p < 0.05$) total richness with 10.0 ± 2.9, 10.0 ± 2.7, and 9.0 ± 1.8, respectively. All hollow fill drainages had significantly lower ($p < 0.05$) EPT richness, %EPT, and %*Ephemeroptera* populations compared to Ref. *Ephemeroptera* populations were entirely absent from the fill drainages except for FMC-D, which was the only fill drainage to have an ephemeropteran taxa collected. *Chironomidae* populations elevated in the fill drainages compared to the reference with FMC-D, TF-D2, and LF-D1 significantly higher ($p < 0.05$) than Ref. Hilsenhoff biotic index was significantly higher in the drainages compared to Ref (Table 1.7). No significant correlation could be established between the benthic community indices and any measured water quality parameter or metal concentration.

Stations below the settling pond in Five Mile Creek reported few significant differences in the benthic macroinvertebrate community. FMC1 had significantly lower ($p < 0.05$) EPT richness compared to FMC2 with 6.8 ± 1.5 and 9.5 ± 1.3, respectively (Table 1.5). Yet, FMC1 had significantly higher %*Ephemeroptera* values compared to

the FMC2 and FMC3 with 6.1 ± 2.0%, 2.5 ± 1.6%, and 2.7 ± 1.5%, respectively. All Five Mile Creek stations below the settling ponds had significantly higher richness, both total and EPT, compared to the hollow fill drainage, FMC-D. Percent *Chironomidae* populations in FMC-D were significantly higher than FMC2 and FMC3 with 53.3 ± 7.5%, 28.5 ± 14.2%, and 24.3 ± 6.1%, respectively.

Upon evaluation of the benthic macroinvertebrate data for the Lavender Fork watershed, there were no significant differences concerning richness, %EPT, %*Ephemeroptera,* or %*Chironomidae* (Table 1.6). Stations below the settling pond did report significant differences in relation to the benthic community of the two hollow fill drainages. Total richness at LF3 was significantly higher than LF-D2 with 14.8 ± 1.7 and 9.0 ± 1.8, respectively. EPT richness at LF2 and LF3 were significantly higher compared to LF-D1 and LF-D2 with values ranging from 2.5 ± 1.0 and 6.0 ± 1.4 at LF-D2 and LF3, respectively. Lastly, %EPT was significantly higher at LF3 compared to LF-D1 with 35.9 ± 4.7% and 8.5 ± 3.2%, respectively.

The downstream stations influenced by the younger fill in Trace Fork (TF1-TF3) had significant differences concerning %EPT where TF1, with 31.7 ± 6.3%, was significantly higher ($p < 0.05$) than TF2 and TF3 with 18.1 ± 1.0% and 15.2 ± 1.4%, respectively (Table 1.6). In contrast TF1 and TF2 had significantly lower %*Chironomidae* populations compared to TF3 with 18.5 ± 1.2%, 19.5 ± 4.6%, and 35.6 ± 6.4%, respectively. The only significant difference between stations the younger hollow fill drainage, TF-D1, and its respective stations below the settling ponds was TF1 and TF2 had significantly higher %EPT populations compared to TF-D1 with 31.7 ± 6.3%, 18.1 ± 1.0%, and 5.8 ± 9.1%, respectively. The were only significantly differences

among the downstream stations influenced by the older hollow fill in the Trace Fork watershed concerned total richness, in which TF-1B was significantly lower than TF-2B with 13.3 ± 1.5 and 17.0 ± 1.4, respectively. The only significant difference concerning the stations below the older settling ponds and the older hollow fill drainage, TF-D2, was TF-2B had significantly higher total richness, with 10.0 ± 2.7 and 17.0 ± 1.4, respectively.

Comparisons were made between the sites below the settling ponds and to the regional reference station, Ref. All Trace Fork sites reported significantly lower EPT richness compared to the reference with values ranging from 2.0 ± 0.8 at both TF1 and TF2 to 7.5 ± 1.0 at Ref (Table 1.6). Both %EPT and %*Ephemeroptera* were significantly lower at stations below the settling ponds compared to the reference. Lastly, all stations below the settling ponds had elevated %*Chironomidae* populations compared to the reference with FMC1, TF3, and all Lavender Fork stations significantly higher than Ref with values ranging from 9.8 ± 3.3% to 53.2 ± 14.9% at Ref and LF2, respectively.

Collector-filterer and shedder functional feeding groups were evaluated at the different stations to study the influence of the potential organic enrichment from the settling ponds. Collector-filterers populations were significantly higher ($p < 0.05$) at stations directly downstream of the ponds, when all stations were grouped according their distance from the ponds (Table 1.7). Collector filterer populations at FMC1 (26.4%) and LF1 (51.4%) were significantly higher than FMC2 (9.2%) and LF2 (18.5%), whereas TF1 (50.7%) was significantly higher than TF3 (19.8%). The shredder communities only had a significant downstream increase in the Five Mile Creek watershed with FMC1 shredders populations significantly lower ($p < 0.05$) than FMC2 and FMC3 with 4.7 ±

1.1%, 12.1 ± 4.9%, and 17.1 ± 8.9%, respectively. Hilsenhoff biotic index (HBI) was lowest in Ref with 1.91 suggesting sensitive taxa were present and dominant, whereas the drainage stations and downstream stations ranged from 4.62 to 6.30 at LF3 and FMC-D suggesting communities of tolerant taxa. No significant relationship could be established be collector filterer populations and chlorophyll *a* concentrations (Table 1.4).

The evaluation of the stations downstream of the settling ponds, grouped according to their location, had significant trends in concerning benthic macroinvertebrate functional feeding groups. First, there was no significant trend or pattern concerning an increase or decrease of total richness, EPT richness, %EPT, %*Ephemeroptera*, or %*Chironomidae* (Table 1.8). Stations closest to the ponds, group1, did have significantly higher collector filterer populations compared to group2 and group3 with 36.4 ± 16.6%, 18.5 ± 9.6%, and 17.1 ± 10.8%, respectively. Shredder communities were significantly lower at group1, which were closer to the ponds, compared to group2 and group3 with 4.6 ± 5.5%, 9.0 ± 7.0%, and 9.1 ± 8.5%, respectively. Among the stations downstream of the ponds, conductivity was found to have a significant negative correlation with %*Ephemeroptera* ($p = 0.0002$) and a positive relationship with %*Chironomidae* ($p = 0.0307$). Water column iron concentrations were found to have a negative relation to total richness ($p = 0.0010$) and EPT richness ($p = 0.0440$). Negatively relationships were established between %*Ephemeroptera* and aluminum ($p = 0.0021$) and copper ($p = 0.0138$) concentrations in the water column.

1.3.6 Habitat Assessment

The average habitat assessment (HA) scores for each station were very similar to stations within the same watershed (Table 1.2). The hollow fill drainages, above the

settling ponds, had habitat condition scores ranging from 118 to 144, out of a possible 200, at LF-D2 and TF-D1, respectively. The major attributes that accounted for the low scores were limited riparian vegetation and streambed substrate for the hollow fill drainages. The habitat scores for the stations downstream of the ponds ranged from 107 to 157.5 at TF3 and LF3, respectively.

1.3.7 *In situ* Asian Clam Testing

Survival and growth data for the *in situ* Asian clam test were analyzed separately for the hollow fill drainages and different watersheds under the assumption each system was not subjected to similar conditions. Therefore, hollow fill drainages and individual stream systems occurring downstream of the settling ponds were analyzed separately, with all stations compared to the regional reference station. Additionally, clam growth was further assessed in a two factor design to evaluate the stations downstream of the ponds in each increment. The data concerning the Asian clam survival and growth are summarized in Table 1.4.

There was no significant mortality in any of the hollow fill drainages (Table 1.4). All of the fill drainages had significantly higher ($p < 0.05$) 60-day growth rates compared to Ref, with average growths of 0.214 ± 0.05 mm and 0.062 ± 0.03 mm, respectively. Upon evaluation of the drainages with the exclusion of the reference station, TF-D1 had significantly higher ($p < 0.05$) growth, 0.279 ± 0.03 mm, than TF-D2 and LF-D1, 0.178 ± 0.04 mm and 0.163 ± 0.02 mm, respectively. Clam growth in the drainages was positively correlated to water column iron concentrations ($p = 0.0442$).

The only significant mortality occurred at LF1, with a 65% *Corbicula* survival rate (Table 1.4). All stations downstream of the ponds, except for LF3, had significantly

higher ($p < 0.05$) growth than the regional reference station (Ref). Evaluation of the clam growth data showed enhanced growth closer to the settlings ponds. For instance, FMC1, LF1, and TF-1B all had significantly better growth ($p < 0.05$) than stations further downstream with 2.41 ± 0.18 mm, 0.773 ± 0.09 mm, and 2.01 ± 0.16 mm respectively. The clam growth for FMC1, LF1, and TF-1B were 30%, 56%, and 47% higher than the next downstream station, respectively. When the stations were grouped according to their relative distance from the ponds, growth at group1 was significantly higher ($p < 0.05$) than group2 and group3 with average growth of 1.6 ± 0.7 mm, 1.0 ± 0.5 mm, and 0.7 ± 06 mm, respectively (Table 1.8). Additionally, group2 was significantly higher than group3 concerning average growth of the clams downstream of the ponds. Clam survival downstream of the ponds were negatively related to water column copper concentrations ($p = 0.0326$), sediment iron levels ($p = 0.0252$), and conductivity ($p = 0.0187$). Clam growth was negatively related to water column manganese concentrations ($p = 0.0094$).

1.3.8 Ecotoxicological Rating

The highest ETR was 86 at Ref, reporting limited environmental stress affecting the station (Table 1.9). ETRs for the hollow fill drainages ranged from 22.3 to 31.3 at LF-D1 and LF-D2, respectively. The stations located downstream of the settling ponds had ETRs ranging from 35.4 to 56.4 at TF3 and FMC2, respectively.

1.4 Discussion

Major coal mining disturbances to stream systems are normally associated with physiochemical alterations as a result of acid mine drainage and habitat disturbance due to stream sedimentation. However, the results of this study suggest only a nominal

impact upon the chemical composition of the water column consisting of elevated conductivity and metal concentrations associated with hollow fill drainages. Conductivity was significantly elevated in the fill drainages compared to the reference station, yet there was a nominal decrease in conductivity in the stations downstream of the settling ponds with increased distance from the ponds. Elevated conductivity, commonly associated with mining influences, ranging between 500 μS/cm and 8000 μS/cm has had significant negative correlations in similar studies with sensitive taxa impairment in the benthic macroinvertebrate community (García-Criado et al. 1999; Soucek et al. 2000; Kennedy et al. 2003). For instance, in the Lavender Fork watershed where conductivity ranged from 2657 μS/cm to 3050 μS/cm, no ephemeropteran taxa were collected. Metal concentrations in the water column, particularly aluminum, were higher in fill drainages and stations downstream of the ponds compared to the regional reference, Ref. It is important to note that aluminum was the only metal concentration elevated above the U. S. EPA's WQC (1999) for aluminum (0.087 mg/L) at all hollow fill drainages and sites downstream of the settling ponds, excluding Five Mile Creek sites. Water column iron concentrations were negatively related to richness, whereas aluminum and copper concentrations were negatively related to *Ephemeroptera* populations. The high water hardness would suggest bioavailable metals would precipitate out, yet there was no evidence of metal precipitate on the substrate, which is known to disrupt suitable habitat thereby hindering macroinvertebrate communities (McKnight and Feder, 1984).

Water column toxicity testing was only significant concerning samples collected from hollow fill drainages in Five Mile Creek and Lavender Fork. The two Lavender

Fork seeps, originating from the same hollow fill, were the only stations that were consistently toxic to *C. dubia*. Lavender Fork fill drainages, LF-D1 and LF-D2, had the elevated aluminum (267 μg/L and 248 μg/L) concentrations (Table 1.3). Soucek et al. (2001b) evaluated aluminum toxicity to *C. dubia* in circumneutral pH levels, however their measured aluminum concentrations were an order of magnitude greater than the levels found the Lavender Fork fill drainages. Additionally, the high water hardness at LF-D1 and LF-D1 (2384 mg/L and 1882 mg/L) would precipitate out bioavailable metals that could be adversely influencing the organisms. However, Stewart et al. (1990) reported that even though high water hardness may protect *Ceriodaphnia* against metal toxicity, it might adversely affect the test organisms as well. For instance, hardness levels 220 mg/L (calcium sulfate) and 1500 mg/L (sodium sulfate) were linked to reduced fecundity rates in *Ceriodaphnia.* The significant mortality in the sediment toxicity test at LF-D2 and FMC2 could not be significantly related to any metal concentration measured for the sediments. Overall, the toxicological data collected did not exhibit good agreement with the benthic macroinvertebrate data, as seen in similar research (Hickey and Clements 1998). For instance, both Lavender Fork drainages were acutely toxic to *C. dubia*, the drainages had the two highest EPT richness averages of the five hollow fill drainages. Additionally, there was significant *Daphnia* mortality in Five Mile Creek, yet *Ephemeroptera* populations, which were almost entirely absent from the other systems, were present and established in the watershed.

One of the major confounding factors of this research was the lack of suitable reference stations due to mining influences. Yet, with only one reference station, the average composition of an uninfluenced headwater stream within the study region was

established. Numerous studies have been conducted on the structure and composition of headwater streams finding leaf-shredding insects are one of the dominant functional feeding group (Short and Maslin 1977; Vannote et al. 1980), which was similar to the conditions within the reference station for our study.

The benthic macroinvertebrate community of the five fill drainages did have reduced richness and sensitive taxa at varying levels of significance, compared to the stations downstream of the ponds. In general, the benthic measurements were lower in the fill drainages compared to the stations downstream of the ponds, except for %*Chironomidae*, which was elevated. Compared to the reference station, the fill drainages had reduced richness, %EPT, %*Ephemeroptera*, and elevated %*Chironomidae*. The elevated HBI values, which evaluate the tolerance of a taxa, and elevated %*Chironomidae* populations suggest a more tolerant population in the fill drainages compared to the reference. Additional support for a more tolerant benthic macroinvertebrate community would be the nearly entire absence of ephemeropteran taxa from the drainages. Mining influences, in similar research, have been related to the shifts in the benthic macroinvertebrate community from sensitive to more tolerant taxa (Leland et al. 1989; Clements, 1994).

In general, the benthic macroinvertebrate community in stations downstream of the ponds did have higher richness and sensitive measurements as well as lower %*Chironomidae* compared to the fill drainages, with varying levels of significance. Additionally, there was little variation among the stations downstream of the ponds within each individual watershed, with limited significant differences. However, upon comparison to the regional references station, each station had lower %EPT and

%*Ephemeroptera*, and Trace Fork had lower EPT richness. Thus, sensitive taxa were reduced in stations downstream of the ponds, compared to the regional reference. Conductivity of the downstream stations was negatively correlated with %*Ephemeroptera* and positively correlated with %*Chironomidae*, suggesting an increase in conductivity could potentially shift the benthic community to more tolerant taxa. HBI values, which are related to benthic macroinvertebrates responds to nutrient and organic pollution, were elevated at sites downstream of the ponds compared to Ref, again suggesting a more tolerant taxa community. Similar studies have suggested that mining disturbances, such as metal inputs and altered water chemistry, shift benthic communities in streams from sensitive based to tolerant based (Leland et al. 1989; Clements et al. 1994).

The most prominent effect reported by the benthic macroinvertebrate communities of the streams studied would be in the composition of the functional feeding groups in stations downstream of the ponds compared to Ref. Normally, headwater streams are primarily a shredder-dominated community (Vannote et al. 1980), as supported by the reference station. Yet, due to the potential organic enrichment originating from the settling ponds, collector filterer populations were well established in stations directly downstream of the ponds. Chlorophyll *a* analysis did not report distinctive differences between fill and downstream stations, however the ponds had high concentrations suggesting abundant algal communities enhancing stations downstream of the ponds. The two factor analysis, evaluating the downstream stations of all watersheds based upon the station's distance from the pond, reported significant alterations in the benthic macroinvertebrate functional feeding groups (Table 1.8). The average collector

filterer populations of the watersheds were significantly elevated at the first station compared to the lower stations. Additionally, the two factor analysis reported shredder communities increasing with distance from the settling pond. Therefore, based upon the functional feeding group alterations, the organic enrichment from the ponds dissipated downstream, with a shredder based community increasing with distance from the ponds.

Corbicula survival was significant at only one station, LF1, immediately below the settling pond in Lavender Fork, which had aluminum and copper concentrations of 223 μg/L and 7.6 μg/L, respectively, within the water column. Studies utilizing Asian clam *in situ* tests (Soucek et al. 2000; Cherry et al. 2002) reported clam mortality associated with these two metals, yet at much higher concentrations. The water hardness of the stations would suggest the metals present did not pose a significant threat to the clams. For instance, a well-established population of *Corbicula* was located in the Lavender Fork watershed, suggesting the clams were able to populate the area with no significant hindrance. The assumption of organic enrichment originating from the settling ponds was also supported by the two factor analysis of the Asian clam growth (Table 1.4). Clam growth was highest at the first downstream stations, with the second and third stations having the second highest and lowest clam growths, respectively (Table 1.8). Thus, growth was greatest closest to the ponds, and significantly decreased with increased distance from the ponds. Again, the chlorophyll *a* analysis suggested abundant algal communities in the settling ponds that could have enhanced the growth rates of clams directly downstream of the ponds. The limited clam growth in Ref is not unusual due to coarse particulate organic matter (CPOM) being the main source of

carbon in headwater streams. Thus, the low growth is best explained by the lack of fine or dissolved organic matter, as seen in similar research (Soucek et al. 2001a).

Evaluation of the benthic macroinvertebrate data and the ETR suggest there was a significant difference between the downstream stations and Ref. Ref was the only station to have an ETR above the 80 percentile, with 86.5. The second highest ETR was 56.4 at FMC2. Therefore, the data utilized in the ETR would suggest significant environmental stress upon all stations except for Ref. The benthic macroinvertebrate data did report sensitive taxa were reduced and more tolerant communities were established in the downstream stations, yet metal concentrations could not be significantly linked to this alteration due to water hardness.

The most significant influence upon the benthic macroinvertebrate community of headwater streams can be attributed to the settling ponds themselves. The assumption of organic enrichment from the ponds was supported by biological data including, elevated collector filterer populations and enhanced clam growth, which would not have occurred in a normal headwater stream as reported by Ref. Chemical analysis of the chlorophyll *a* concentrations did not suggest significant alterations in the streams, however the ponds had high concentrations. Yet, the argument of the settling ponds being a detrimental influence upon the stream systems is double sided. For instance, as shown in Table 1.3, the settling ponds play an integral role in lowering the water column metal concentrations originating from the hollow fill drainages. The absence of the settling ponds would allow for unimpeded flow of the fill drainages into the headwater streams, which could inhibit sensitive species such as *Ephemeroptera* that were clearly reduced in these systems.

In conclusion, the influences originating from the hollow fills did not significantly hinder the benthic macroinvertebrate populations within the stations downstream of the settling ponds. Although, the downstream stations did differ significantly from the regional reference station in the composition of taxa present due to the potential organic enrichment from the settling ponds. The ponds received runoff from the hollow fills, which are normally heavy fertilized upon completion, inputting nitrogen and phosphorous into the ponds contributing to a large algal community and organic enrichment of the downstream stations. Yet, upon assessing the stations downstream of the ponds at the different increments, the influences of the organic enrichment for the ponds dissipated downstream as supported by the decrease in collector filterer populations and clam growth. In contrast, shredder populations increased with greater distance from the ponds suggesting the benthic communities in the streams were shifting to a composition similar to the reference.

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Figure 1.1. Map of the study area indicating West Virginia streams utilized for the research. Lavender Fork (LF; n=5) in Boone County and Trace Fork (TF; n=7) and Five Mile Creek (FC; n=4) in Mingo County. A regional reference station was located in Five Mile Creek watershed as well.

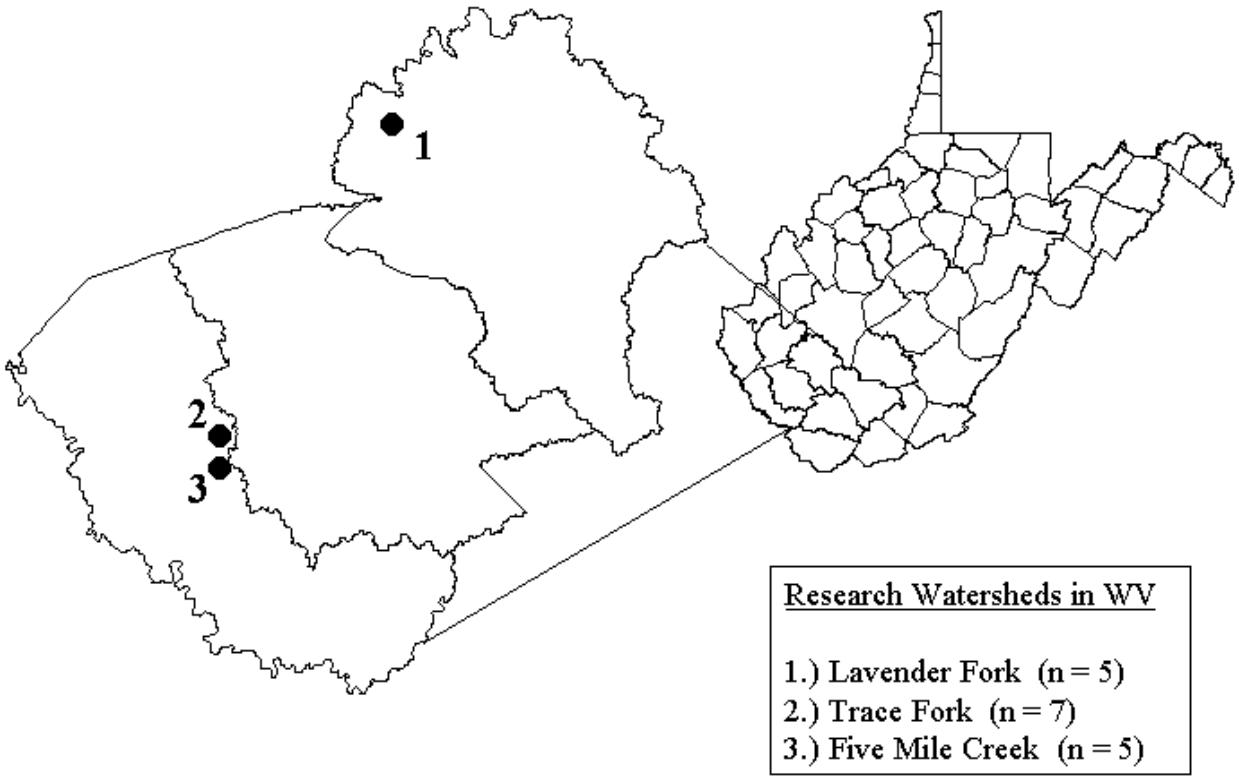

Table 1.1. Categorical division of the research stations in Five Mile Creek (Mingo Cty.), Trace Fork (Mingo Cty.), and Lavender Fork (Boone Cty.) in West Virginia, USA.

Station	Station Type	Description
Ref	Reference Site	Regional reference site above mining influence in Five Mile Creek
FMCD	Hollow Fill Drainage	Drainage originating from a 7 year old hollow fill within Five Mile Creek
FMC 1, 2 & 3	Downstream Sites	Research stations downstream of the settling pond at the toe of the Five Mile Creek hollow fill
TF-D1	Hollow Fill Drainage	Drainage originating from a 3 year old hollow fill within Trace Fork
TF 1, 2, & 3	Downstream Sites	Research stations downstream of the settling pond at the toe of the 3 yrs. old Trace Fork hollow fill
TF-D2	Hollow Fill Drainage	Drainage originating from a 15 year old hollow fill within Trace Fork
TF 1B-2B	Downstream Sites	Research stations downstream of the settling pond at the toe of the 15 yrs. old Trace Fork hollow fill
LF-D1	Hollow Fill Drainage	Left Drainage originating from a 6 year old hollow fill within Lavender Fork
LF-D2	Hollow Fill Drainage	Right Drainage originating from a 6 year old hollow fill within Lavender Fork
LF1, 2, & 3	Downstream Sites	Research stations downstream of the settling pond at the toe of the Lavender Fork hollow fill

Table 1.2. Mean water quality data (± standard deviation) for the influence of hollow fill drainages upon headwater streams in WV. Parameters are divided into the reference station, drainages originating from the hollow fills, and stations 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.					
	pH	**Conductivity**	**Alkalinity**	**Hardness**	**Habitat**
Reference Station		µS/cm	mg/L as $CaCO_3$		
Ref	7.2 ± 0.36	247 ± 87	72 ± 52	86± 20	152.0
Hollow Fill Drainages					
FMC-D	7.42 ± 0.20	1557 ± 544*	328 ± 88*	993 ± 343*	132.0
TF-D1	7.84 ± 0.13	1310 ± 323	240 ± 24	743 ± 123	144.0
TF-D2	7.98 ± 0.09	1643 ± 370*	352 ± 35*	1078 ± 182*	127.5
LF-D1	8.09 ± 0.03	3050 ± 883*	239 ± 23	2384 ± 678*	126.0
LF-D2	7.93 ± 0.18	2497 ± 780*	296 ± 77*	1882 ± 707*	118.0
Downstream Stations					
FMC1	8.02 ± 0.14	991 ± 421	200 ± 70	551 ± 233	138.5
FMC2	8.37 ± 0.47*	965 ± 420	196 ± 70	557 ± 232	147.0
FMC3	8.14 ± 0.21*	923 ± 380	216 ± 95	544 ± 226	117.5
TF1	8.13 ± 0.03*	1248 ± 323	222 ± 20	699 ± 121	146.0
TF2	8.15 ± 0.03*	1231 ± 312	222 ± 30	710 ± 129	142.5
TF3	8.15 ± 0.08*	1200 ± 288	217 ± 26	687 ± 121	107.0
TF-1B	8.13 ± 0.06*	1355 ± 352	265 ± 37*	855 ± 204*	134.0
TF-2B	8.22 ± 0.06*	1310 ± 327	258 ± 34	819 ± 196*	119.5
LF1	8.09 ± 0.05	2720 ± 929*	217 ± 62	1904 ± 596*	130.0
LF2	8.10 ± 0.06	2667 ± 939*	218 ± 59	1830 ± 526*	140.0
LF3	8.11 ± 0.08*	2657 ± 956*	218 ± 63	1812 ± 554*	157.5

* Denotes significant differences from the reference station

Table 1.3. Metal concentrations for the influence of hollow fill drainages upon headwater streams in WV. Parameters are divided into the reference station, drainages originating from the hollow fills, and stations downstream of the settling ponds associated with the hollow fill.

Metal Concentrations for West Virginia Hollow Fill Study Stations								
Sites	**Al-Water**	**Fe-Water**	**Cu-Water**	**Mn-Water**	**Al-Sed**	**Fe-Sed**	**Cu-Sed**	**Mn-Sed**
	mg/L	mg/L	mg/L	mg/L	mg/kg	mg/kg	mg/kg	mg/kg
Reference Station								
Ref	0.022	0.013	bd	0.04	10.57	50.5	0.018	1.42
Hollow Fill Drainages								
FMC-D	0.137 [a]	0.026	0.002	0.092	8.30	79.8	0.019	1.68
TF-D1	0.129 [a]	0.072	0.004	0.053	10.61	65.9	0.040	2.25
TF-D2	0.147 [a]	0.016	0.003	0.111	22.83	112.7	0.103	7.86
LF-D1	0.267 [a]	0.015	0.009	0.027	16.99	114.5	0.060	9.73
LF-D2	0.248 [a]	0.020	0.008	0.321	13.73	185.0	0.048	82.1
Downstream Stations								
FMC1	0.079	0.016	0.001	0.007	8.69	48.6	0.015	2.52
FMC2	0.080	0.012	0.002	0.01	15.27	70.1	0.025	3.05
FMC3	0.079	0.022	bd	0.032	8.80	48.5	0.012	1.56
TF1	0.118 [a]	0.055	0.004	0.021	16.47	66.6	0.048	5.43
TF2	0.116 [a]	0.060	0.004	0.017	15.33	70.9	0.037	3.87
TF3	0.113 [a]	0.149	0.004	0.014	19.30	85.7	0.058	3.4
TF-1B	0.120 [a]	0.014	0.004	0.006	18.14	104.8	0.052	7.86
TF-2B	0.118 [a]	0.008	0.003	0.007	15.95	86.5	0.046	5.61
LF1	0.223 [a]	0.038	0.008	0.030	19.63	105.3	0.046	16.12
LF2	0.225 [a]	0.036	0.006	0.046	20.17	157.6	0.122	17.1
LF3	0.144 [a]	0.034	0.005	0.019	16.69	138.3	0.040	7.75

[a] Denotes Concentrations that exceed the Continuous Criteria
(U.S. EPA National Recommended Water Quality Criteria)
bd denotes metal concentrations below the detection limit

Table 1.4. Chlorophyll *a* concentration measurements for fill drainages, settling ponds, and stations downstream of the ponds. Mean Asian clam growth (± standard deviation) and mean survival rates are reported below.

***In situ* Asian clam toxicological data and chlorophyll *a* concentrations.**					
Sites	**Chlorophyll *a***	**60-Day Growth**		**Survival**	
	mg/m^3	**mm**		**%**	
Ref	0.32	0.06 ± 0.03		100 ± 0	
FMC-D	3.51	0.23 ± 0.04	AB	100 ± 0	A
TF-D1	1.87	0.28 ± 0.03	A	95 ± 10	A
TF-D2	1.39	0.18 ± 0.04	BC	100 ± 0	A
LF-D1	1.04	0.16 ± 0.02	C	100 ± 0	A
LF-D2	1.55	0.22 ± 0.03	AB	100 ± 0	A
FMC Pond	11.25				
FMC1	2.28	2.42 ± 0.18	A	95 ± 10	A
FMC2	3.71	1.68 ± 0.17	B	100 ± 0	A
FMC3	2.25	0.46 ± 0.14	C	100 ± 0	A
TF Pond1	4.78				
TF1	1.70	1.29 ± 0.16	AB	100 ± 0	A
TF2	0.38	1.01 ± 0.03	B	85 ± 19	A
TF3	4.60	1.46 ± 0.33	A	95 ± 10	A
TF Pond2	12.92				
TF-1B	1.03	2.01 ± 0.06	A	95 ± 10	A
TF-2B	1.77	1.06 ± 0.16	B	95 ± 10	A
LF Pond	8.45				
LF1	1.55	0.77 ± 0.09	A	65 ± 10	B
LF2	1.92	0.34 ± 0.11	B	85 ± 19	AB
LF3	1.95	0.17 ± 0.14	B	95 ± 10	A

Stations with different letters are significantly different from one another.

Table 1.5. Toxicological results concerning acute water column toxicity testing involving *Ceriodaphnia dubia* and sediment toxicity testing involving *Daphnia magna*. Mean results and standard deviations are reported.

Research Stations	*Ceriodaphnia dubia* LC50 (%)	*Daphnia magna* Sediment Toxicity Testing Survival (%)		Reproduction	
Reference Station					
Ref	n/a	100 ± 0.0	A	41.4 ± 1.4	AB
Hollow Fill Drainages					
FMC-D	70.71	83.3 ± 8.3	AB	34.9 ± 13.4	AB
TF-D1	n/a	91.7 ± 4.2	AB	41.4 ± 8.0	AB
TF-D2	n/a	100 ± 0.0	A	62.3 ± 5.0	A
LF-D1	71.63 ± 0.80	93.3 ± 4.2	AB	36.9 ± 1.8	AB
LF-D2	70.63 ± 23.1	33.3 ± 9.5	B	16.8 ± 18.6	B
Downstream Stations					
Five Mile Creek					
FMC1	n/a	75 ± 8.0	AB	23.6 ± 8.8	B
FMC2	n/a	33.3 ± 9.5	B	18.0 ± 17.4	B
FMC3	n/a	75 ± 12.5	AB	27.6 ± 18.9	AB
Trace Fork					
TF-1	n/a	83.3 ± 8.3	AB	36.9 ± 11.8	AB
TF-2	n/a	100 ± 0.0	A	40.8 ± 1.8	AB
TF-3	n/a	100 ± 0.0	A	37.1 ± 9.0	AB
TF-1B	n/a	100 ± 0.0	A	37.5 ± 4.9	AB
TF-2B	n/a	100 ± 0.0	A	41.4 ± 4.3	AB
Lavender Fork					
LF1	n/a	100 ± 0.0	A	39.5 ± 2.7	AB
LF2	n/a	75 ± 12.5	AB	30.5 ± 20.8	AB
LF3	100	100 ± 0.0	A	35.1 ± 7.9	AB

Stations with different letters are significantly different from each other.

Table 1.6. Mean benthic macroinvertebrate data (± standard deviations) concerning hollow fill influences within West Virginia headwater streams. Comparisons in each watershed were made between the fill drainages and sites below the ponds as well as between the reference station and each individual system and fill drainage category.

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	
Hollow Fill Drainages										
FMC-D	11.8 ± 2.2	A	2.0 ± 0.8	A*	5.8 ± 3.0	A*	0.63 ± 1.2	A*	53.3 ± 7.5	A*
TF-D1	10.0 ± 2.9	A*	1.0 ± 1.4	A*	5.8 ± 9.1	A*	0	A*	32.0 ± 15.1	A
TF-D2	10.0 ± 2.7	A*	1.8 ± 1.7	A*	19.3 ± 22.2	A*	0	A*	41.5 ± 19.6	A*
LF-D1	10.5 ± 2.4	A	3.0 ± 0.8	A*	8.5 ± 3.2	A*	0	A*	49.3 ± 11.2	A*
LF-D2	9.0 ± 1.8	A*	2.5 ± 1.0	A*	23.8 ± 18.0	A*	0	A*	30.8 ± 14.8	A
Downstream Stations										
Five Mile Creek										
FMC1	19.5 ± 2.1	A†	6.8 ± 1.5	B†	17.4 ± 3.1	A*	6.1 ± 2.0	A*†	35.6 ± 9.6	A*
FMC2	21.5 ± 3.3	A†	9.5 ± 1.3	A†	18.3 ± 4.0	A*	2.5 ± 1.6	A*	28.5 ± 14.2	A†
FMC3	18.8 ± 1.0	A†	7.3 ± 1.0	AB†	28.5 ± 16.5	A*†	2.7 ± 1.5	A*	24.3 ± 6.1	A†
Trace Fork										
TF-1	10.3 ± 2.2	A*	2.8 ± 1.5	A*	31.7 ± 6.3	A*†	2.0 ± 1.7	A*	18.5 ± 1.2	B
TF-2	11.3 ± 1.3	A	2.0 ± 0.8	A*	18.1 ± 1.0	B*†	0	A*	19.5 ± 4.6	B
TF-3	10.8 ± 2.2	A	2.0 ± 0.8	A*	15.2 ± 1.4	B*	1.7 ± 2.0	A*	35.6 ± 6.4	A*
TF-1B	13.3 ± 1.5	B	3.3 ± 1.0	A*	19.5 ± 6.6	A*†	0	A*	29.7 ± 12.1	A
TF-2B	17.0 ± 1.4	A†	4.0 ± 1.4	A*	23.1 ± 8.8	A*	0	A*	33.5 ± 7.9	A
Lavender Fork										
LF1	12.3 ± 2.4	A	4.8 ± 1.0	A	32.3 ± 8.3	A*	0	A*	35.6 ± 7.7	A*
LF2	12.5 ± 1.7	A	5.5 ± 1.3	A†	32.3 ± 15.9	A*	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*

* represents a significant difference between stations and the reference station.
† represents a significant difference between stations below ponds and respective fill drainage(s).
Stations with different letters are significantly different from one another.

Table 1.7. Mean benthic macroinvertebrate functional feeding groups (± standard deviations) and Hilsenhoff's Biotic Index to assess tolerance of a benthic community.

Stations	%Collector Filterers		%Shredders		HBI
Reference Station					
Ref	0.19 ± 0.4		49.9 ± 6.2		1.91
Hollow Fill Drainages					
FMC-D	3.2 ± 3.2	A*	5.1 ± 4.3	AB*	6.30
TF-D1	7.2 ± 10	A*	17.2 ± 11.7	AB*	5.82
TF-D2	2.7 ± 3.1	A*	16.2 ± 18.8	AB*	6.03
LF-D1	24.4 ± 3.4	A*	0.33 ± 0.4	B*	5.68
LF-D2	2.3 ± 1.6	A*	4.2 ± 3.7	AB*	5.46
Downstream Stations					
Five Mile Creek					
FMC1	26.4 ± 7.4	A*	4.7 ± 1.1	B*	5.70
FMC2	9.2 ± 2.2	B*	12.1 ± 4.9	A*	5.34
FMC3	5.8 ± 4.5	B*	17.1 ± 8.9	A*	5.10
Trace Fork					
TF-1	50.7 ± 4.5	A*	3.9 ± 5.1	A*	5.76
TF-2	28 ± 10.4	AB*	7.4 ± 1.8	A*	5.59
TF-3	19.8 ± 7.8	B*	9.5 ± 4.5	A*	6.06
TF-1B	17.2 ± 7.2	B*	9.6 ± 7.1	A*	5.64
TF-2B	18.5 ± 4.4	B*	15.8 ± 7.0	A*	5.53
Lavender Fork					
LF1	51.4 ± 7.9	A*	0.3 ± 0.4	A*	5.64
LF2	18.5 ± 9.4	B*	0.85 ± 0.1	A*	5.01
LF3	25.7 ± 8.2	B*	0.75 ± 0.9	A*	4.62

* represents a significance difference between stations and the reference station.
Stations with different letters are significantly different from one another.

Table 1.8. Evaluation of stations downstream of the settling ponds, grouped according to their distance from the pond. Group1 consisted of stations directly downstream of the ponds (ex. FMC1), Group2 consisted of stations downstream of the first (ex. FMC2), and Group3 consisted of the lowest stations in the watersheds (ex. FMC3). Mean benthic macroinvertebrate and clam data and standard deviations are reported.

Benthological and toxicological measurements evaluating stations downstream of settling ponds in distance increments.

	Total Richness		EPT Richness		%EPT		%Ephemeroptera	
Group1	13.8 ± 4.0	A	4.4 ± 2.0	B	25.2 ± 9.1	A	2.1 ± 2.9	A
Group2	15.6 ± 4.6	A	5.3 ± 3.0	A	23.0 ± 10.2	A	0.6 ± 1.3	B
Group3	14.8 ± 3.7	A	5.1 ± 2.5	AB	26.5 ± 12.7	A	1.5 ± 1.7	AB

	%Chironomidae		%Collector-Filterers		%Shredders		Clam Growth	
Group1	29.9 ± 10.6	A	36.4 ± 16.6	A	4.6 ± 5.5	B	1.6 ± 0.7	A
Group2	33.7 ± 16.2	A	18.5 ± 9.6	B	9.0 ± 7.0	A	1.0 ± 0.5	B
Group3	32.3 ± 9.0	A	17.1 ± 10.8	B	9.1 ± 8.5	A	0.7 ± 0.6	C

Groups with different letters are significantly different from one another.

Table 1.9. Ecotoxicological rating (ETR) procedure results.

Research Stations	ETR Scores
Reference Station	
Ref	86.5
Hollow Fill Drainages	
FMC-D	29.6
TF-D1	29.0
TF-D2	30.1
LF-D1	22.3
LF-D2	31.3
Downstream Stations	
Five Mile Creek	
FMC1	55.3
FMC2	56.4
FMC3	49.2
Trace Fork	
TF-1	42.9
TF-2	37.4
TF-3	35.4
TF-1B	41.3
TF-2B	41.1
Lavender Fork	
LF1	39.4
LF2	38.7
LF3	42.4

2 Coal generated hollow fill drainages and post-mining influences upon the Middle Creek watershed, VA USA.

Abstract. The Middle Creek watershed at Cedar Bluff in southwest Virginia, USA, was evaluated for potential influences originating from head of hollow fills constructed from excess spoil of surface coal mining. Bioassessment techniques, including water/sediment chemistry, acute water column toxicity testing with *Ceriodaphnia dubia*, chronic sediment toxicity testing with *Daphnia magna*, *in situ* Asian clam (*Corbicula fluminea* [Müller]) toxicity testing, and benthic macroinvertebrate surveys, were utilized in evaluating the environmental influences of hollow fill and abandoned mined land drainages upon the stream biota. Conductivity in the watershed ranged from 286 ± 95 μS/cm to 778 ± 75 μS/cm at reference and hollow fill related sites. Dissolved water column metal and sediment concentrations were elevated at sites associated with hollow fill and abandon mined land influences compared to a upstream reference site. Asian clam growth was significantly reduced ($p < 0.05$) at sites associated with the hollow fill drainages, which were not impeded by holding or settling ponds before discharging into Middle Creek. Benthic macroinvertebrate surveys were conducted in 2001 and 2002 with little significant differences reported for 2001. The benthic macroinvertebrate indices for 2002 had reduced total richness, EPT (*Ephemeroptera-Plecoptera-Trichoptera*) richness and %EPT in a site associated with a recently constructed hollow fill drainage and reclaimed abandoned mined land. Overall, the benthic macroinvertebrate communities of the Middle Creek watershed were not significantly influenced by the hollow fill and post-mining influences. Conductivity was significantly negatively related to total richness ($r = -0.744$, $p = 0.0087$) and EPT richness ($r = -0.736$, $p = 0.0099$) for the Middle Creek watershed in 2002.

2.1 Introduction

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). Similar work has been conducted in the western United States (Chadwick and Canton, 1983), Canada (Grout and Levings, 2001), Europe (García-Criado et al. 1999), and Japan (Watanbe et al. 2000). However, little research has been focused upon the long-term implications of surface coal mining produced hollow fills and associated drainages upon low order streams.

Coal mining has been a major industry in the Appalachian region since the introduction of the railroad to the area in the 1880's (Hibbard, 1987). The subject of concern for this research is the influence hollow fills have upon a low order stream in southwestern VA. Hollow fills are the byproduct of surface coal mining, which includes area, contour and mountaintop mining. The fills are comprised of excess spoil and debris that is not returned to the original mining site for disposal (Fung, 1981). Therefore, the material is normally placed in nearby or adjacent hollows that may influence nearby low order (first-third order) streams, especially in mountainous regions similar to the Appalachian region.

Numerous studies have evaluated the environmental impacts associated with mine drainages, as well as the negative effects upon stream biota, such as benthic macroinvertebrates. 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 (Courtney and Clements, 1998; Guerold et al. 2000). In addition to lowered pH, elevated conductivity is common with land disturbances and mine drainages. Soucek et al. (2000) reported elevated conductivity suggested fewer taxa present at those sites, whereas Kennedy et al. (2003) found sensitive aquatic fauna were impaired at an approximate conductivity of 3700 μS/cm in a study assessing the effect of an active mine drainage upon a watershed in Ohio. Heavy metal influx and precipitation, as a result of severe

mine drainage, are major detrimental factors to benthic macroinvertebrate communities. For instance, heavy metal pollution has been responsible for shifts in the benthic communities from sensitive to more tolerant taxa (Clements, 1994) as well as smothering of habitat due to metal precipitation (McKnight and Feder, 1984).

The focus of this study was an evaluation of hollow fill and abandoned mined land drainages upon the benthic macroinvertebrate communities of the Middle Creek watershed in southwestern Virginia. The Middle Creek watershed was subjected to postmining influences originating from three major hollow fills and an abandoned coal processing plant (ACPP) and associated settling ponds. Land disturbances occurred during the two-year study at the start of the second sampling season (Winter 2001-2002) due to reclamation processes that removed the ACPP and ponds as well as a settling pond at the toe of a hollow fill. We employed bioassessment techniques including water column/sediment chemistry, benthic macroinvertebrate sampling, toxicity testing with *Ceriodaphnia dubia* and *Daphnia magna*, and *in situ* toxicity testing with the Asian clam (*Corbicula fluminea* [Müller]). The purpose of the study was to determine the physiochemical alterations in the water quality due to the hollow fill drainages, as well an assessment of the benthic macroinvertebrate communities to evaluate whether the potential influences constituted an impact upon those populations.

2.2 Study Sites

The Middle Creek watershed is located outside of Cedar Bluff, VA, USA (Fig. 2.1). It is a tributary of the Clinch River, which is of particular interest due to recent declines of native unionid species within the Cumberland plateau (Diamond et al. 2002). Focus was placed on three hollow fills and an abandoned mined land (AML) area within the Middle Creek watershed. The uppermost hollow fill was constructed nine yrs. ago, with a continuous flowing drainage into

a settling pond before discharging into Middle Creek. The second and third hollow fills, eight and fourteen yrs. old, respectively, drain directly into Middle Creek during rain events, and are assumed to influence Middle Creek with subsurface drainages. Three settling ponds and respective drainages near the abandoned coal processing plant (ACPP) were evaluated for potential influence on Middle Creek. The ACPP area minimally influenced two Middle Creek sites (MCAP1 and MCAP2) adjacent to the AML area and runoff collected in the settling ponds, which discharged above MCPD. Lastly, three sites were established in the Clinch River, into which Middle Creek flows, to evaluate Middle Creek's immediate influence upon the river. Site descriptions and postmining influences are summarized in Table 2.1.

Lands adjacent to Middle Creek were disturbed in the late winter of 2001-2002 due to land reclamation processes. The abandoned coal processing plant and associated settling ponds were removed and the land was reclaimed. Additionally, the topography of the upper most fill was altered to ensure better drainage and the settling pond below it was removed. Therefore, the hollow fill's drainage flowed unimpeded into Middle Creek upstream of its previous position. Due to these alterations within the watershed, some site locations were removed and additional locations added in the second season, detailed in Table 2.1.

2.3 Methods

2.3.1 Water Column & Sediment Chemistry

Water samples were collected seasonally, n = 7, (2001-2002) with water quality parameters measured each trip. Conductivity and pH measurements were conducted under field conditions and the water 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 the protocols by the Standard Methods for the Examination of Water and Wastewater (APHA 1995). Additionally, water and sediment samples were collected on one occasion for metal analysis at the Inductively Coupled Plasma Spectrometry (ICP) lab at Virginia Tech. Water samples were filtered (pore size 0.47 μm) and analyzed for dissolved metal concentrations of aluminum (Al), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Sediment samples were digested using (1+1) nitric acid and (1+4) hydrochloric acid under reflux heating according to U.S. EPA protocols (1991). The digested samples were then submitted to the ICP laboratory for analysis for total metal concentrations of Al, Cu, Fe, Mn, and Zn.

2.3.2 Water Column Toxicity Testing

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

2.3.3 Sediment Toxicity Testing

Sediment samples were collected in February 2002 and August 2002 for chronic toxicity testing using the cladoceran, *Daphnia magna*. *Daphnia* were cultured by personnel of the Aquatic Ecotoxicology lab at Virginia Tech using filtered Sinking Creek water at the culture water. Sinking Creek water 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 $CaCO_3$, respectively. Sediment samples were collected using a clean polypropylene scoop per site and placed in Ziploc® freezer bags, and stored at 4 °C for no longer than two weeks. Tests were conducted in accordance to the procedures of 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 produced for each 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. Sediment from the reference stations within the Middle Creek watershed and the Clinch River were utilized as controls.

2.3.4 Benthic Macroinvertebrate Survey

Benthic macroinvertebrate surveys were conducted in June 2001 and May 2002 in accordance to the U.S. EPA Rapid Bioassessment Protocols (RBPs) (Barbour et al. 1999). A D-frame dipnet, with an 800-µm mesh net, was utilized in conducting qualitative samples per 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 including richness, *Ephemeroptera-Plecoptera-Trichoptera* (EPT) richness, %EPT, %*Ephemeroptera*, and %*Chironomidae*.

2.3.5 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 an overall maximum score of 200. Therefore, the higher the score 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 were responsible for any measured stress within the watershed.

2.3.6 *In situ* Asian clam toxicity testing

Asian clams (*Corbicula fluminea* [Müller]) were collected from a reference site in the New River near Ripplemead, VA with clam rakes and returned to the Ecosystem Simulation Laboratory (ESL) at Virginia Tech and temporary stored in a Living Stream® (Frigid Units, Toledo, OH). 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 each were placed in polyurethane mesh bags, measuring 18 cm by 36 cm with 0.5 cm^2 openings, and transported to the sites. Four replicates (20 clams) were fastened and arranged around a piece of rebar and placed in riffle habitats at each research sites. The Asian clam *in situ* test occurred in summer 2002, after the reclamation processes within the watershed. Survival and growth were measured every 30 days for an approximate 60-

day test period (May 14 thru July 15, i.e. 62 days). Asian clam mortality was assessed as individuals with valves either gapping open or easily teased apart.

2.3.7 Ecotoxicological Rating Procedure

An ecotoxicological rating (ETR) system was developed in order to better evaluate the environmental conditions at the sites within the study. The ETR utilized in this study was based on that implemented in southwestern VA in similar research (Soucek et al. 2000; Cherry et al. 2001). Ecological, toxicological, and physiochemical data collected for the study were selected for analysis due the particular parameter's relevance to mine drainage influences upon aquatic systems. The ecological measurements of total taxa richness, EPT richness, %EPT, %*Ephemeroptera*, and %*Chironomidae* were selected due to the sensitivity or tolerance of the parameter. Transplanted Asian clam (*C. fluminea*) growth was selected as the toxicology measure due to growth sensitivity in the presence of mining toxicants (Grout and Levings 2001). Also, physicochemical parameters, such as conductivity, dissolved Al and Cu concentrations in the water column, and habitat analyses, were selected for relevance to the post-mining conditions within the Middle Creek watershed.

All parameters, except conductivity, %*Chironomidae*, and dissolved Al and Cu, were transformed into a percentage with the highest value measured equaling 1 and subsequent values were then divided by the highest value (value measured at a site/highest value measured in the watershed). Conductivity and %*Chironomidae* measurements were transformed into a percentage with the lowest value measured equaling 1 and subsequent values were then used to divide the lowest value (lowest value measured in the watershed/value measured at a site). Dissolved Al and Cu concentrations for each site were assessed with values under water quality criteria receiving a 1.0 and values over criteria receiving a 0.1. The ecological parameters of

total richness, EPT richness, %*Ephemeroptera*, and %EPT were multiplied by 12.5 due to their ecological sensitivity. The parameters of %*Chironomidae* and conductivity were multiplied by 10, and dissolved Al and Cu, habitat assessment, and Asian clam growth were multiplied by 7.5. The highest cumulative score a site could obtain would be 100. The ETR works on a basis of the higher the total score, the less environmentally impacted the site. Cherry et al. (2001) developed a percentile ranking whereby sites scoring 90% were excellent, 80% were acceptable, 70% were marginal, and ≤ 60% were stressed.

2.3.8 Statistical Analyses

Data including water quality analysis, sediment toxicity testing, macroinvertebrate indices, Asian clam endpoints, and habitat assessment, were analyzed in the Statistical Analysis System (SAS Institute). Data were tested for normality using the Shapiro-Wilks test ($\alpha = 0.05$) within SAS. Comparisons of the normally and non-normally disturbed data were made between the sites using the Tukey-Kramer honestly significant difference post-hoc test ($\alpha = 0.05$) and Wilcoxon Rank sum in SAS. 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® software (Sall and Lehman 1996).

2.4 Results

2.4.1 Water Column and Sediment Chemistry Results

Conductivity in the upper hollow fill drainage, D1, with 778 ± 75 μS/cm, was significantly higher ($p < 0.05$) than the four most upstream sites within Middle Creek, MCRF1, MCRF2, MCUP and MCBP with conductivity ranging from 286 ± 95 μS/cm at MCRF1 to 340 ± 173 μS/cm at MCUP (Table 2.2). A water hardness of 405 ± 11 mg/L as $CaCO_3$ at D1, was significantly higher ($p < 0.05$) than all Middle Creek mainstem sites (Table 2.2). The hardness at

MCRF1 was significantly different from MC/CR with 45 ± 12 mg/L as $CaCO_3$ and 183 ± 52 mg/L as $CaCO_3$, respectively. The pH for the Middle Creek sites ranged between 7.7 ± 0.35 and 8.2 ± 0.08 at MC-HF4 and MC/CR, respectively. The highest metal concentrations within the streambed sediment were located in the hollow fill drainage, D1 (Table 2.2). No significant differences were found in any of the water quality parameters measured for the ACPP settling ponds. Water quality parameters measured in the Clinch River had no significant differences among the sites (Table 2.2). Conductivity within the Clinch River sites ranged between 299 ± 41μS/cm and 337 ± 80 μS/cm at CROMC and CRMC, respectively. There were no significant trends in the dissolved water column and sediment metal concentrations in the Clinch River sites to suggest metal input from the Middle Creek watershed (Table 2.3).

2.4.2 Water Column Toxicity Testing

Water column toxicity testing involving *C. dubia*, occurred in 2001 (summer, fall, and winter) and 2002 (seasonally). Toxicity was only associated with the settling ponds located around the abandoned coal processing plant (ACPP) in 2001. Ponds 1,2, & 3, produced LC_{50}s of 5.03, 1.41, and 5.03 % of sample water, respectively (Table 2.4). The toxicity of the pond water increased after rain events and decreased to no evident toxicity under drought conditions, suggesting the toxicant source originated from the surrounding landscape that was scoured after rain events. Surface materials with apparent toxic potentials, such as coal processing spoil and debris, were removed from the area along with the settling ponds during land reclamation in early winter 2002 which prevented any further toxicity testing. No Middle Creek or Clinch River site reported any toxicity to *C. dubia* during 2001 or 2002.

2.4.3 Sediment Toxicity Testing

The first round of sediment toxicity testing in February 2002, before reclamation processes were completed, reported no significant differences for survival or fecundity among the Middle Creek sites. Lowest *Daphnia* survival was 77.8 ± 38.5% for both MCBP and MC-HF1 (Table 2.4). The *Daphnia* fecundity rates for Middle Creek ranged between 63.1 ± 7.5 and 39.1 ± 13.2 at MCRF1 and MC-HF1, respectively. The sediment collected from the sites within the Clinch River had no significant differences for *Daphnia* survival or fecundity.

The second sediment toxicity test, in August 2002 after reclamation processes were completed, reported significant mortality ($p < 0.05$) at MCAP1 and MC-HF4, with survival rates of 41.7 ± 15.1% and 33.3 ± 13.9%, respectively (Table 2.4). *Daphnia* fecundity was significantly reduced ($p < 0.05$) at MCAP1 and MC-HF4 with 17.9 ± 7.1 and 15.0 ± 18.3, respectively. The overall highest fecundity occurred at MC-HF1 with 44.1 ± 5.8. Sediment collected from the Clinch River sites sampled in Fall 2002 reported no significant mortality or reproductive impairment. No significant correlations could be established between mortality and impaired fecundity with any sediment metal concentration.

2.4.4 Benthic Macroinvertebrate Sampling

Total taxa richness and EPT (*Ephemeroptera*, *Plecoptera*, *Trichoptera*) richness were highest at the upstream reference site, MCRF1, with 23.0 ± 4.1 and 12.3 ± 1.3 in 2001, and 27.8 ± 1.3 and 16.8 ± 0.5 in 2002, respectively (Table 2.5). Total richness and EPT richness were lowest at MCAP2 in 2001 (dropped in 2002) with 8.8 ± 3.4 and 5.0 ± 2.7, respectively, and D1 in 2002 (added in 2002) with 7.0 ± 1.6 and 3.3 ± 1.3, respectively. Percent EPT varied greatly during the two years with no significant differences for the 2001 sampling season ranging between 71.4 ± 14.7% and 38.1 ± 13.4% at MCRF1 and MC/CR, respectively. In contrast,

MC/CR had the highest %EPT in 2002 with 85.7 ± 3.8%, whereas D1 reported the lowest %EPT of 34.1 ± 14.3%. Percent *Ephemeroptera* was highest in 2001 at MCAP2 with 44.7 ± 6.5% and in 2002 at MC/CR with 75.23 ± 6.3%. The lowest %*Ephemeroptera* was at MC-HF3 in 2001 and D1 in 2002 with 9.1 ± 3.6% and 10.7 ± 13.2%, respectively. Percent *Chironomidae* was highest in 2001 at MCHF-3 with 47.5 ± 15.4% and in 2002 at D1 with 35.6 ± 9.6%. Conductivity was found to have a significant negative relationship with total richness ($p = 0.0087$) and EPT richness ($p = 0.0099$) (Table 2.6). Dissolved Mn concentrations were negatively related to total richness, EPT richness, and %EPT, and positively related to the more tolerant %*Chironomidae* index. Metal concentrations measured in the streambed sediment produced significant negative correlations ($p < 0.05$) for total richness, EPT richness, and %EPT, and significant positive correlations ($p < 0.05$) with %*Chironomidae.*

Total richness measurements in the Clinch River were highest in 2001and 2002 at the reference site, CRRF, with 24.5 ± 4.8 and 31.0 ± 2.3, respectively (Table 2.5). No significant differences occurred for EPT richness in 2001, however in 2002 CRRF was significantly higher ($p < 0.05$) than both CROMC and CRMC with 17.3 ± 1.3, 10.5 ± 4.2, and 11.0 ± 1.4, respectively. Percent EPT and %*Ephemeroptera* were lowest in both years at CRRF with 26.2 ± 8.9% and 16.0 ± 6.3%, respectively, in 2001 and 46.1 ± 11.7% and 36.7 ± 12.8%, respectively, in 2002. There were no significant differences concerning %*Chironomidae* in either 2001 or 2002 among any of the sites. There were no significant differences among the benthic macroinvertebrate data between CROMC and CRMC for either 2001 or 2002 sampling season.

2.4.5 Habitat Assessment

Habitat assessment was conducted in June 2002 by two researchers with the averaged scores recorded and analyzed. The hollow fill drainage, D1, had the lowest habitat score of 77.5

due to bank erosion and limited riparian vegetation zone (Table 2.2). MCPD was the highest scored site, with 141.5, in the Middle Creek watershed. The remaining sites ranged between 102.5 and 139 at MCAP1 and MCUP, respectively. The Clinch River sites all scored relatively high with 144.5 and 141.5 for CRRF and CROME/CRME, respectively. No significant correlation between habitat conditions and benthic macroinvertebrate impairment could be established (Table 2.6).

2.4.6 *In situ* Asian clam toxicity testing

No significant mortality occurred in the *in situ* Asian clam toxicity test (Table 2.3). The lowest survival score for the 62-day period was 90 ± 20% at MC-HF4. Clam growth, however, was significantly reduced at multiple locations in the Middle Creek watershed. MCRF1, the upstream reference site, had the highest clam growth of 1.19 ± 0.18 mm (Table 2.3). Sites associated with the hollow fill drainages, MC-HF1-MC-HF4, had significantly reduced ($p < 0.05$) Asian clam growth measurements of 0.85 ± 0.07 mm, 0.65 ± 0.04 mm, 0.55 ± 0.03 mm, and 0.80 ± 0.11 mm, respectively, compared to MCRF1. Clam growth at MCUP and MCPD were significantly lower ($p < 0.05$) than the upstream reference station, MCRF1, with values of 0.98 ± 0.11 mm and 0.89 ± 0.06 mm, respectively. The lowest overall *Corbicula* growth, 0.08 ± 0.01 mm, occurred in the hollow fill drainage, D1. No significant differences were reported concerning *Corbicula* survival and growth among the Clinch River sites. Clam growth within the Clinch River ranged between 1.71 ± 0.21 mm and 1.19 ± 0.24 mm at CROMC and CRMC, respectively. No significant relationships could be established between Asian clam survival or growth against any metal concentration or measured water quality parameter.

2.4.7 Ecotoxicological Rating

The three uppermost sites within the watershed, MCRF1, MCRF2, and MCUP, scored highest for the ETRs, with 82.7, 85.0, and 84.2, respectively (Table 2.7). The lowest ranking site was the hollow fill drainage, D1, with a 36.7 score, suggesting a stressed environment. The ETRs for the remaining sites ranged between 79.5 and 68.6 at MC-HF1 and MCPD, respectively. The Clinch River sites scored between 85 and 90.1 at CRMC and CROMC, respectively, suggesting no abnormal stress upon the sites.

2.5 Discussion

The overall water quality of Middle Creek was not severely influenced by the drainages originating from the hollow fills located within the watershed. The lowest average pH for Middle Creek was 7.7 ± 0.35 at MC-HF4, which well above the acidic pH normally associated with acid mine drainage. For instance, Cherry et al. (2001) recorded pH as low as 2.73 in AMD research conducted in Lee County, VA, USA. Conductivity for the all of the Middle Creek mainstem sites, including hollow fill and AML influenced sites, were not significantly different. Only the hollow fill drainage, D1, was significantly different from the reference and upstream sites within the watershed. Since elevated conductivity is commonly associated with mining activities, as seen in similar research (Soucek et al. 2000; Kennedy et al. 2003), it has been proven to be an excellent indicator of mining related influences. For instance, García-Criado et al. (1999) conducted research on the performance of some abiotic and biotic indices in rivers affected by coal mining in Spain. Correlation analysis demonstrated that sulfate and conductivity, ranging from 20 μS/cm to 449 μS/cm, were most related to biological parameters in indicating mining impacts.

The water column toxicity testing involving *Ceriodaphnia* provided little information concerning the overall environmental conditions of the watershed due to limited mortality. The only significant mortality was associated with the ACPP area that was removed in early 2002. The landscape surrounding the ACPP area was reclaimed thereby removing the problematic settling ponds and associated toxic constituents tied up in the landscape. Therefore, the absence of toxicity upon *Ceriodaphnia* at the remaining sites within the watershed suggests the discharges originating from the hollow fill posed little or no acute toxicological impairment upon the benthic macroinvertebrate communities present.

The sediment toxicity tests, involving *D. magna*, evaluated chronic exposure of sediment toxics, mainly metal constituents. The February 2002 test had no significant difference for either survival or fecundity suggesting no impairment within the watershed for that particular time period. However, the September 2002 sediment test had significant differences in both *Daphnia* survival and fecundity. The lowest *Daphnia* survival for the Sept. 2002 test was MC-HF4, which is directly downstream of the last hollow fill in the watershed. However, metal concentrations associated with the sediment at MC-HF4 were lower than the three sites upstream associated with the hollow fills, MC-HF1, MC-HF2, and MC-HF3, which reported no mortality. Therefore, the data, as supported by no significantly negative relationships, suggest the impairment constituent(s) were not related to the metal concentrations within the sediments. It is important to point out the overall fecundity rate for the *Daphnia* in February and September were 53 and 34, respectively. Viganò (2000) conducted 7-day sediment toxicity tests involving *Ceriodaphnia* with sediments from the River Po in Italy during the winter and summer seasons. Viganò reported results similar to those mentioned above, with greater survival and fecundity rates occurring in the winter and reduced values associated with the summer sediment test.

Viganò found that the seasonal sediments were significantly different with variations in the oxygen demands and C/N ratios between the two seasons, yet no significant differences concerning the organic carbon of the whole sediment or fine materials were found. It has been established that organic carbon content has been significantly related to toxicity with *D. magna*, *Hyalella azteca*, and *Chironomus tentans* (Meyer et al. 1993). Even though our results were very similar to the outcome of Viganò's test, we can only speculate upon the C/N ratios and organic content of the sediments in the Middle Creek study. However, the data suggest metal concentrations in the sediments were not linked to any significant *Daphnia* impairment.

Research conducted by Soucek et al. (2001) and Grout and Levings (2001) reported the effectiveness of bivalve species as indicators of mining impacts upon benthic communities. The primary endpoint of the Asian clam test was survival, which proved to be a significant indicator in AMD-impacted streams (Soucek et al. 2001), was not significant within the Middle Creek watershed. The lowest survival rate was 90%, associated with site MC-HF3, which was in no manner effective in distinguishing between different levels of impacts found in Middle Creek. Clam growth however, as a secondary endpoint, proved to be a more sensitive measure for the conditions found in the Middle Creek watershed. The lowest growth within the watershed was in the hollow fill drainage, D1, which was not unusual due to limited food and nutrient availability in the drainage. Clam growth at sites associated with the land reclamation of the ACPP and lower hollow fills (MCPD, MC-HF1– MC-HF4) did have significantly reduced growth compared to the upstream reference sites. However, no significant relationship could be established between any abiotic parameter, such as conductivity or metal concentrations, and reduced clam growth rates (Table 2.6) for the Middle Creek watershed.

Evaluation of the benthic macroinvertebrate community for the two years had differences between the two sampling periods, particularly richness (Table 2.5). The 2001 benthic macroinvertebrate sampling was conducted in June under normal base flow conditions, whereas the 2002 sampling was conducted in May under low flow conditions due to drought conditions affecting the entire region. However, the time variation between the sampling periods best explains the alterations in the benthic macroinvertebrate richness due to taxa collected in 2002 that were absent in 2001. The earlier sampling period in 2002 (May) collected taxa that missed in 2001 (June) due to early emergence patterns.

The first benthic macroinvertebrate sampling data (2001) had little significant differences among the sites within the Middle Creek watershed. Conditions within Middle Creek were supportive for high taxa richness as supported by data collected at the upstream reference station, MCRF1. The lowest richness for 2001 occurred at MCAP2, which however contained the highest %*Ephemeroptera* among all sites for that time period. Numerous field studies have reported ephemeropteran populations as a sensitive measure of metal and environmental stressors (Beltman et al. 1999; Kiffney and Clements, 1993; Soucek et al. 2001). MCAP2 was adjacent to the ACPP and received minimal runoff from the area, however the causative agents for the low taxa richness were undetermined. The reduced sensitive ephemeropterans and high tolerant *Chironomidae* population at MC-HF3 suggested an environmental impact, which did have the second highest streambed sediment metal concentrations for the watershed. A positive relationship between %*Chironomidae* and sediment metal concentrations (Table 2.6) provided support that the more tolerant species were able to proliferate whereas the more sensitive taxa were inhibited, as supported by Richardson and Kiffney (2000).

In the 2002 benthic macroinvertebrate survey, the upstream reference station, MCRF1, once again contained the highest richness for the watershed, suggesting no alteration to the upstream section of the creek. The lowest overall macroinvertebrate scores were for the upper hollow fill drainage, D1. The drainage was a product of reclamation processes within the watershed in late 2001/early 2002. Therefore, the reduced taxa richness and benthic indices in the drainage are best explained by the poor habitat conditions and limited colonization time. Total and EPT richness were reduced at MCPD compared to the two upstream reference sites, which was directly downstream of the land reclamation to the ACPP. There were no significant qualities about MCPD to relate to the poor richness, other than the land disturbances and reclamation. The hollow fill drainage, D1, had reduced *Ephemeroptera* taxa and elevated *Chironomidae* taxa, suggesting a more tolerant community. The Middle Creek / Clinch River confluence site, MC/CR, which had the second lowest total and EPT richness, had the highest %EPT and %*Ephemeroptera* within the watershed for the 2002 sampling season, with the majority of the population consisting of *Baetidae* taxa. Kiffney and Clements (1993) studied bioaccumulation of heavy metals in benthic macroinvertebrates and found that members of the *Baetidae* family were sensitive indicators of metal contamination. Therefore, *Baetidae* presence suggested metal contamination was not the detrimental factor resulting in the low richness. MC/CR did have poor substrate (Table 2.2) and was downstream of a residential area, both of which could have negatively contributed to the lower richness, however there is no supportive data for that assumption.

Significant negative and positive relationships were established between benthic macroinvertebrate indices and abiotic parameters, such as conductivity and metal concentrations (Table 2.6). The negative relationship between total and EPT richness with conductivity is

supported by similar research reporting similar results (García-Criado et al. 1999; Soucek et al. 2000; Kennedy et al. 2003), suggesting higher conductivity allows fewer taxa. Overall, the more sensitive taxa, including richness and %EPT, were significantly negatively correlated with the metal concentrations whereas the more tolerant taxa, %*Chironomidae*, was significantly positively correlated. Dissolved manganese concentrations in the water column were significantly correlated with benthic indices, however concentrations were low enough not to pose a hindrance to the benthic community. Although, %*Chironomidae* was positively related with metal concentrations that were negatively related with reduced sensitive taxa, the index did not provide a definitive measure of environmental stress in the watershed. For example, the %*Chironomidae* for the upstream reference site, MCRF1, was not significantly different from many of the mining influenced sites for the 2002 sampling period.

Overall, the hollow fill drainages did not severely influence the benthic macroinvertebrate communities within Middle Creek. In 2002, benthic macroinvertebrate richness and sensitive taxa were reduced at sites associated with the land reclamation and recently constructed hollow fill drainage compared to the reference sites. Water quality was altered, with significant negative relationships established between taxa richness and EPT richness against conductivity and sediment metal concentrations, yet the negative relationships were directly linked to the upper hollow fill drainage. The low significant relationship between measured metals and conductivity against the more sensitive indices of %EPT and %*Ephemeroptera* suggest measured physicochemical parameters were not the major hindering factor within the watershed. Toxicity testing, involving *C. dubia* and *D. magna* provided little insight due to limited toxicity or seasonal alterations in impairment. *Corbicula* toxicity testing reported reduced growth at sites associated with hollow fill drainages and reclaimed lands

compared to reference sites, however no significant correlation could be established to attribute a factor for the reduced growth. The ecotoxicological rating procedure suggested that the hollow fill influences did not significantly impair the watershed with only one site scoring below the 60th percentile, which was the upper hollow fill drainage due to its recent construction and poor habitat conditions. Additionally, as supported by the ETRs and benthic macroinvertebrate data, the Middle Creek watershed had no immediate discernible influence upon the Clinch River with little alterations occurring between sections of the river receiving Middle Creek drainage and the section uninfluenced by Middle Creek.

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Figure 2.1. Research sites located within the Middle Creek watershed outside of Cedar Bluff, Tazewell County, VA, USA. Sites with an † were removed and sites with a * were added in 2002 sampling season due to reclamation processes in the watershed.

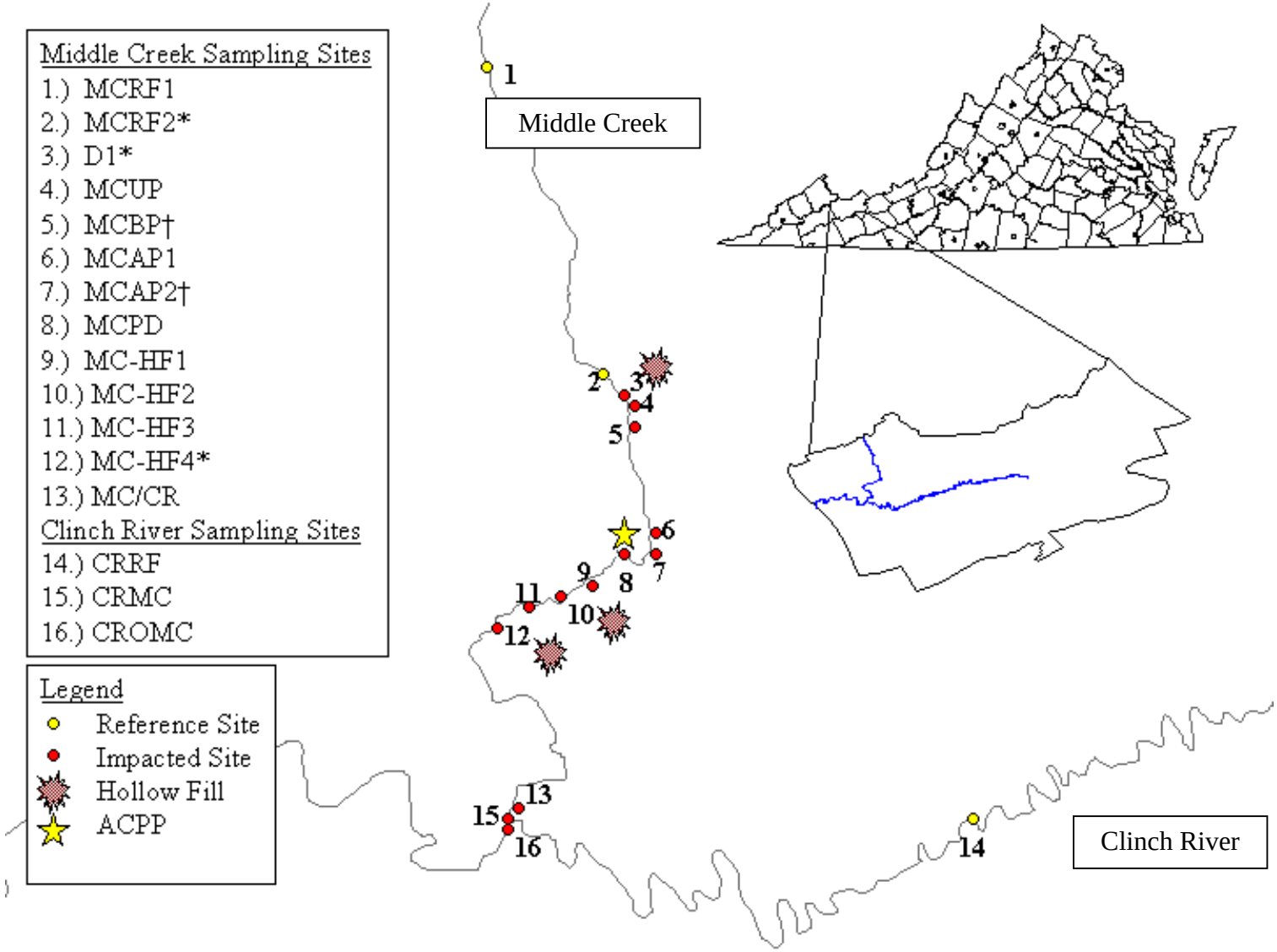

Table 2.1. Sites are listed for the Middle Creek watershed, Clinch River, and settling ponds associated with the abandoned coal processing plant (ACPP). Alterations within the watershed are denoted with an * for sites added or a †for deleted sites due to reclamation processes.

Sites	Comments
Middle Creek	
MCRF1	Upstream reference site
MCRF2*	Reference site above first hollow fill (added in 2002)
D1*	Drainage originating from the upstream 9 yrs old hollow fill
MCUP	Site upstream of hollow fill drainage (2001) and then downstream of the drainage in 2002 due to reclamation processes
MCBP†	Site downstream of hollow fill drainage in 2001, removed in 2002
MCAP1	Site adjacent to the ACPP area
MCAP2†	Site adjacent to the ACPP (removed in 2002 due to altered habitat)
MCPD	Downstream ACPP pond drainages
MC-HF1	Adjacent to 8 yrs old fill
MC-HF2	Adjacent to 8 yrs old fill
MC-HF3	Adjacent to 14 yrs old fill
MC-HF4	Adjacent to 14 yrs old fill (added in 2002)
MC/CR	Confluence of Middle Creek and Clinch River
Clinch River	
CRRF	Upstream reference site
CROMC	Site opposite Middle Creek
CRMC	Site downstream Middle Creek's confluence
Coal Plant Ponds (ACPP)	
Pond1†	First pond in a series of three
Pond2†	Second pond in a series of three
Pond3†	Third and final pond, drained into Middle Creek

Table 2.2. Mean water quality data (± standard deviation) and habitat assessment scores (HAS) for the Middle Creek watershed and Clinch River, Tazewell Cty., VA, USA.

Water Quality and Habitat Data for Middle Creek and Clinch River, VA, USA

	pH		Conductivity		Alkalinity		Hardness		HAS
Middle Creek Sites			µS/cm		mg/L as $CaCO_3$				
MCRF1	8.2 ± 0.19	A	286 ± 95	A	132 ± 34	A	45 ± 12	A	117.0
MCRF2	8.0 ± 0.12	A	303 ± 120	A	125 ± 41	A	85 ± 58	AB	128.5
D1	8.2 ± 0.10	A	778 ± 75	B	212 ± 7	A	405 ± 11	C	77.5
MCUP	8.0 ± 0.25	A	340 ± 173	A	108 ± 56	A	114 ± 100	AB	139.0
MCBP	7.98 ± 0.45	A	243 ± 46	A	68 ± 11	A	71 ± 1.4	AB	n/a
MCAP1	8.0 ± 0.29	A	464 ± 108	AB	120 ± 36	A	159 ± 79	AB	102.5
MCAP2	8.01 ± 0.11	A	480 ± 71	AB	105 ± 38	A	115 ± 21	AB	n/a
MCPD	8.0 ± 0.06	A	466 ± 103	AB	118 ± 30	A	163 ± 79	AB	141.5
MC-HF1	8.1 ± 0.17	A	430 ± 78	AB	106 ± 31	A	166 ± 64	AB	136.0
MC-HF2	8.1 ± 0.16	A	437 ± 92	AB	105 ± 26	A	166 ± 58	AB	129.0
MC-HF3	7.9 ± 0.40	A	596 ± 348	AB	154 ± 85	A	173 ± 53	AB	130.5
MC-HF4	7.7 ± 0.35	A	515 ± 123	AB	148 ± 48	A	175 ± 37	AB	128.0
MC/CR	8.2 ± 0.08	A	562 ± 145	AB	145 ± 33	A	183 ± 52	B	105.5
ACPP Settling Ponds									
Pond1	7.97 ± 0.10	A	603 ± 68	A	75 ± 28	A	226 ± 79	A	n/a
Pond2	7.92 ± 0.20	A	657 ± 71	A	55 ± 20	A	268 ± 86	A	n/a
Pond3	7.99 ± 0.20	A	537 ± 52	A	83 ± 13	A	205 ± 34	A	n/a
Clinch River Sites									
CRRF	8.14 ± 0.20	A	305 ± 34	A	150 ± 8.7	A	152 ± 15	A	144.5
CROMC	8.26 ± 0.10	A	299 ± 41	A	143 ± 16	A	147 ± 8.4	A	141.5
CRMC	8.28 ± 0.21	A	337 ± 80	A	142 ± 14	A	139 ± 30	A	141.5

Mean values are listed for the water quality data with significance listed beside the parameter (sites with different letters are statistically significant from each other). Habitat assessment scores were generated in the late 2002 summer.

Table 2.3. Dissolved water column and streambed sediment metal concentrations for the Middle Creek watershed and Clinch River sampling sites.

Metal Concentrations for Middle Creek & Clinch River, Tazewell Cty. VA (USA).										
Sites	**Al-Water**	**Fe-Water**	**Cu-Water**	**Mn-Water**	**Zn-Water**	**Al-Sed**	**Fe-Sed**	**Cu-Sed**	**Mn-Sed**	**Zn-Sed**
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Middle Creek Sites										
MCRF1	0.027	0.1221	0.0055	0.0138	0.0024	22.39	58.4	0.0304	1.679	0.198
MCRF2	<0.0038	0.1924	0.001	0.0155	0.0079	17.26	57.6	0.0315	1.088	0.179
D1	<0.0038	0.0686	0.0018	0.2087	0.0033	60.6	257.3	0.1722	8.51	0.715
MCUP	0.0147	0.08	0.0048	0.0155	0.0105	30.23	117.1	0.0678	3.689	0.347
MCBP	0.019	0.0802	0.0047	0.008	0.011	n/a	n/a	n/a	n/a	n/a
MCAP1	0.0288	0.2375	0.0091	0.0807	0.0104	46.71	101.4	0.088	3.069	0.408
MCAP2	0.0296	0.2409	0.0044	0.0758	0.0146	n/a	n/a	n/a	n/a	n/a
MCPD	0.0249	0.2568	0.0035	0.0735	0.0124	23.73	68.4	0.0429	1.208	0.292
MC-HF1	0.0247	0.27	0.0034	0.0423	0.0086	29.3	94.1	0.0776	2.891	0.404
MC-HF2	0.0232	0.2492	0.0069	0.0406	0.0084	23.67	65.5	0.0418	1.539	0.309
MC-HF3	0.0211	0.3142	0.0062	0.0442	0.007	31.52	112.6	0.0634	4.706	0.424
MC-HF4	0.0154	0.4227	0.0036	0.0772	0.0078	18.96	61.2	0.0278	0.609	0.239
MC/CR	0.0194	0.1507	<0.001	0.0266	0.0045	26.84	98.6	0.066	2.174	0.400
Clinch River Sites										
CRRF	0.0095	0.0422	0.0024	0.0038	0.0034	34.1	105.9	0.065	4.97	0.342
CROMC	0.0058	0.0381	<0.001	0.0028	0.0049	23.54	99.2	0.081	1.84	0.403
CRMC	0.0194	0.0659	0.0022	0.007	0.0054	26.42	136.8	0.094	2.33	0.918

Table 2.4. Toxicological results for the Middle Creek, ACPP settling ponds, and Clinch River sites are summarized below. Mean and standard deviation values for water column (WC) and sediment toxicity testing, and *in situ* Asian clam toxicity testing are listed. Sites lost or added due to the reclamation processes in Middle Creek have dashes designating no data was collected for that site.

	WC Testing		**Sediment Toxicity Testing**				**Sediment Toxicity Testing**				***In situ* Asian Clam Test**			
	2001	**2002**	**February 2002**				**September 2002**				**2002**			
Sites	**LC_{50} (%)**		**Survival (%)**		**Reproduction**		**Survival (%)**		**Reproduction**		**Survival (%)**		**Growth (mm)**	
MCRF1	n/a	n/a	100 ± 0.0	A	63.1 ± 7.5	A	100 ± 0.0	A	42.6 ± 5.1	A	95 ± 10	A	1.19 ± 0.18	A
MCRF2	-	n/a	-	-	-	-	100 ± 0.0	A	31.8 ± 6.6	ABC	95 ± 10	A	1.09 ± 0.10	AB
D1	-	n/a	-	-	-	-	83.3 ± 6.9	ABC	34.6 ± 3.3	ABC	100 ± 0	A	0.08 ± 0.11	F
MCUP	n/a	n/a	100 ± 0.0	A	62.1 ± 5.2	A	100 ± 0.0	A	43.6 ± 3.0	A	100 ± 0	A	0.98 ± 0.01	BC
MCBP	n/a	-	77.8 ± 38.5	A	52.4 ± 12.5	A	-	-	-	-	-	-	-	-
MCAP1	n/a	n/a	100 ± 0.0	A	53.9 ± 5.6	A	41.7 ± 15.1	BC	17.9 ± 7.1	C	95 ± 10	A	1.14 ± 0.05	A
MCAP2	n/a	-	100 ± 0.0	A	59.3 ± 7.1	A	-	-	-	-	-	-	-	-
MCPD	n/a	n/a	100 ± 0.0	A	52.7 ± 6.7	A	100 ± 0.0	A	40.1 ± 5.9	AB	100 ± 0	A	0.89 ± 0.06	CD
MC-HF1	n/a	n/a	77.8 ± 38.5	A	39.1 ± 13.2	A	100 ± 0.0	A	44.1 ± 5.8	A	95 ± 10	A	0.85 ± 0.07	CD
MC-HF2	n/a	n/a	100 ± 0.0	A	55.1 ± 3.7	A	100 ± 0.0	A	40.3 ± 5.2	AB	95 ± 10	A	0.65 ± 0.04	DEF
MC-HF3	n/a	n/a	88.9 ± 19.2	A	49.2 ± 13.7	A	100 ± 0.0	A	33.3 ± 6.3	ABC	90 ± 20	A	0.55 ± 0.03	EF
MC-HF4	n/a	n/a	100 ± 0.0	A	45.6 ± 4.4	A	33.3 ± 13.9	C	15.0 ± 18.3	BC	95 ± 10	A	0.80 ± 0.11	CDE
MC/CR	n/a	n/a	100 ± 0.0	A	46.9 ± 7.8	A	75 ± 18.0	AB	27.8 ± 18.6	ABC	100 ± 0	A	1.07 ± 0.08	AB
Pond 1	5.03	-	-	-	-	-	-	-	-	-	-	-	-	-
Pond 2	1.41	-	-	-	-	-	-	-	-	-	-	-	-	-
Pond 3	5.03	-	-	-	-	-	-	-	-	-	-	-	-	-
CRRF	n/a	n/a	77.8 ± 19.2	A	35.9 ± 3.2	A	100 ± 0.0	A	23.1 ± 7.6	A	100 ± 0	A	1.26 ± 0.12	A
CROMC	n/a	n/a	88.9 ± 19.2	A	40.7 ± 8.9	A	100 ± 0.0	A	23.8 ± 5.1	A	100 ± 0	A	1.71 ± 0.21	A
CRMC	n/a	n/a	100 ± 0.0	A	53.1 ± 9.5	A	75 ± 18.0	A	19.7 ± 9.1	A	95 ± 10	A	1.19 ± 0.24	A

Sites with different letters are significantly different ($p < 0.05$) from each other.

Table 2.5. Mean (± standard deviation) benthic macroinvertebrate data for the June 2001 and May 2002 sampling periods. Sites containing a dash were not sampled for that particular time period.

Sites	Total Richness		EPT Richness		%EPT		%Ephemeroptera		%Chironomidae	
	2001		**2001**		**2001**		**2001**		**2001**	
Middle Creek Sites										
MCRF1	23.0 ± 4.1	A	12.3 ± 1.3	A	71.4 ± 14.7	A	26.1 ± 10.8	ABC	12.2 ± 9.8	ABC
MCRF2	-	-	-	-	-	-	-	-	-	-
D1	-	-	-	-	-	-	-	-	-	-
MCUP	16.5 ± 4.4	AB	9.5 ± 4	AB	57.6 ± 10.6	A	14.5 ± 6.5	BC	22.5 ± 6.9	ABC
MCBP	19.0 ± 10.9	AB	10.0 ± 5.7	AB	62.1 ± 11.0	A	10.0 ± 3.5	C	8.2 ± 5.5	BC
MCAP1	14.0 ± 5.6	AB	5.8 ± 2.9	AB	43.3 ± 4.3	A	10.0 ± 8.1	C	16.3 ± 9.3	ABC
MCAP2	8.8 ± 3.4	B	5.0 ± 2.7	B	60.3 ± 21.1	A	44.7 ± 6.5	A	26.2 ± 21.1	ABC
MCPD	17.3 ± 2.8	AB	8.8 ± 1.7	AB	54.2 ± 11.7	A	22.4 ± 5.7	ABC	4.8 ± 3.7	C
MC-HF1	16.8 ± 2.1	AB	6.3 ± 1.3	AB	52.4 ± 14.3	A	16.0 ± 10.9	BC	7.7 ± 1.5	BC
MC-HF2	16.5 ± 3.9	AB	6.8 ± 2.1	AB	41.7 ± 11.8	A	14.7 ± 2.1	BC	17.3 ± 19.1	ABC
MC-HF3	15.5 ± 1.9	AB	7.3 ± 0.5	AB	42.1 ± 15.5	A	9.1 ± 3.6	C	47.5 ± 15.4	A
MC-HF4	-	-	-	-	-	-	-	-	-	-
MC/CR	17.0 ± 2.6	AB	6.5 ± 1.0	AB	38.1 ± 13.4	A	28.0 ± 9.4	AB	27.6 ± 5.7	AB
Clinch River Sites										
CRRF	24.5 ± 4.8	A	9.8 ± 2.1	A	26.2 ± 8.9	B	16.0 ± 6.3	B	16.3 ± 1.9	A
CROMC	23.3 ± 4.0	AB	10.5 ± 2.9	A	56.6 ± 6.2	A	52.4 ± 7.0	A	20.2 ± 4.2	A
CRMC	15.8 ± 4.7	B	7.5 ± 2.4	A	60.7 ± 20.0	A	55.3 ± 17.1	A	16.9 ± 15.1	A
Sites	**Total Richness**		**EPT Richness**		**%EPT**		**%Ephemeroptera**		**%Chironomidae**	
	2002		**2002**		**2002**		**2002**		**2002**	
Middle Creek Sites										
MCRF1	27.8 ± 1.3	A	16.8 ± 0.5	A	74.3 ± 2.3	ABCD	36.2 ± 9.7	BC	15.4 ± 1.0	BCDE
MCRF2	25.8 ± 2.2	AB	15.3 ± 1.9	AB	77.4 ± 3.4	ABCD	40.3 ± 9.3	ABC	7.5 ± 1.3	EF
D1	7.0 ± 1.6	E	3.3 ± 1.3	E	34.1 ± 14.3	D	10.7 ± 13.2	C	35.6 ± 9.6	A
MCUP	20.7 ± 3.2	BCD	14.0 ± 2.6	ABC	84.6 ± 4.6	AB	39.8 ± 12.7	ABC	5.7 ± 1.0	F
MCBP	-	-	-	-	-	-	-	-	-	-
MCAP1	19.0 ± 2.2	CDE	12.5 ± 0.6	BCDE	70.8 ± 7.5	BCD	46.8 ± 4.3	AB	21.4 ± 4.2	AB
MCAP2	-	-	-	-	-	-	-	-	-	-
MCPD	20.3 ± 3.4	CD	11.3 ± 2.1	CDE	59.0 ± 15	CD	37.0 ± 12.1	BC	19.2 ± 7.0	ABC
MC-HF1	21.3 ± 2.1	ABC	13.5 ± 1.3	ABC	82.3 ± 4.6	ABC	46.7 ± 8.2	AB	8.3 ± 2.7	CDEF
MC-HF2	22.3 ± 2.1	ABC	13.5 ± 1.3	ABC	80.7 ± 4.6	ABC	45.7 ± 8.0	AB	8.9 ± 2.7	DEF
MC-HF3	21.5 ± 1.3	BC	14.3 ± 1.3	ABC	75.3 ± 8.8	ABCD	31.9 ± 4.2	BC	18.2 ± 7.5	ABCD
MC-HF4	20.8 ± 2.6	BCD	14.3 ± 1.7	ABC	76.2 ± 9.0	ABCD	35.0 ± 9.4	BC	18.8 ± 6.8	ABC
MC/CR	13.0 ± 0.8	DE	8.3 ± 1.0	DE	85.7 ± 3.8	A	75.2 ± 6.3	A	6.1 ± 2.2	F
Clinch River Sites										
CRRF	31.0 ± 2.3	A	17.3 ± 1.3	A	46.1 ± 11.7	B	36.7 ± 12.8	B	19.5 ± 11.3	A
CROMC	19.0 ± 6.8	B	10.5 ± 4.2	B	69.6 ± 7.5	A	64.5 ± 9.7	A	15.1 ± 3.9	A
CRMC	20.5 ± 4.1	B	11.0 ± 1.4	B	68.8 ± 9.6	AB	63.2 ± 9.9	AB	19.4 ± 7.5	A

Sites with different letters signify a significant difference between the sites.

Table 2.6. Results from correlation analysis evaluating abiotic parameters and biotic indices generated for the 2002 sampling season.

	Total Richness	EPT Richness	% EPT	% Ephemeroptera	% Chironomidae	Clam Growth
Dissolved Al	0.39	0.429	0.433	0.44	-0.223	0.248
Dissolved Cu	0.346	0.422	0.159	-0.04	0.116	-0.068
Dissolved Fe	0.386	0.483	0.393	0.13	-0.134	0.003
Dissolved Mn	-0.682*	-0.615*	-0.627*	0.355	0.818*	-0.336
Dissolved Zn	0.215	0.229	0.174	0.121	-0.161	-0.206
Sediment Al	-0.759*	-0.743*	-0.730*	-0.447	0.736*	-0.192
Sediment Cu	-0.727*	-0.752*	-0.355	-0.064	0.227	-0.318
Sediment Fe	-0.747*	-0.747*	-0.427	-0.318	0.382	-0.536
Sediment Mn	-0.546	-0.51	-0.391	-0.318	0.273	-0.391
Sediment Zn	-0.865*	-0.850*	-0.666*	-0.368	0.619*	-0.363
Conductivity	-0.744*	-0.736*	-0.544	-0.351	0.489	-0.443
PH	-0.323	-0.446	-0.2	0.155	-0.067	0.119
Habitat Assessment	0.296	0.197	-0.088	-0.203	0.047	0.143

Values marked with an asterisk (*) denote a significant correlation ($p < 0.05$) for the particular relationship.

Table 2.7. Ecotoxicological rating (ETR) system scores for the Middle Creek sites of 2002. ETR scores below the 60th percentile suggest environment stress at the particular sites.

Sites	ETR
MCRF1	82.7
MCRF2	85.3
D1	36.7
MCUP	84.3
MCAP1	69.5
MCPD	68.6
MC-HF1	79.5
MC-HF2	77.4
MC-HF3	69.2
MC-HF4	71.4
MC/CR	78.0
CRRF	86.0
CROMC	90.1
CRMC	85.0

3 Evaluation of surface coal mining spoil generated hollow fill drainages upon two streams in southwest Virginia

Abstract. Drainage influences originating from four hollow fills constructed from surface coal mining spoil were assessed in the South Fork of the Pound River (SFPR) and Powell River watersheds in southwestern Virginia, USA. Bioassessment procedures included water/sediment analysis, water column and sediment toxicity testing with *Ceriodaphnia dubia* and *Daphnia magna*, benthic macroinvertebrate surveys, and *in situ* toxicity testing with Asian clams (*Corbicula fluminea* [Müller]). Hollow fill drainages had elevated conductivity and water column/sediment metal concentrations compared to receiving stream sites in the SFPR and Powell River watersheds. Metal concentrations were lower in sites below settling ponds compared to hollow fill drainages, which were above the ponds, possibly due to dilution and precipitation. Fill drainages and an AMD influenced tributary in the SFPR watershed were acutely toxic to *Ceriodaphnia* with no significant *Daphnia* mortality in sediment toxicity testing. Asian clam toxicity testing had significant mortality associated with one fill drainage and the AMD influenced tributary in the SFPR. Clam growth was enhanced downstream of the settling ponds due to potential organic enrichment from algal growth in the ponds. In both watersheds, the lowest benthic macroinvertebrate richness observations were recorded in the hollow fill drainages. *Ephemeroptera* populations were absent from the SFPR watershed, with no significant impairment in the Powell River receiving stream. Conductivity was negatively correlated to *Ephemeroptera-Plecoptera-Trichoptera* (EPT) richness and a modified EPT ratio, EPT-*Hydropsychidae*, in the SFPR ($p = 0.023$, $p = 0.013$), yet no relationship was established in the Powell River watershed.

3.1 Introduction

Several studies have been conducted upon aquatic systems within the Appalachian region addressing concerns including industrial discharges (Farris et al. 1998), surface coal mining (Matter and Ney, 1981), acid mine drainage, (Soucek et al. 2001a; Cherry et al. 2001), and land usage (Diamond et al. 2002). The Appalachian region has been an area of concern due to the decline of native mussel species in recent years (Sheehan et al. 1989; Diamond et al. 2002). Coal mining is a major industry within the region, with drainages and discharges originating from mining sites regulated by the Clean Water Act (1977) and Surface Mining Control and Reclamation Act of 1977. The water quality and structure of low order streams play an integral role in the quality and integrity of larger systems downstream (Vannote et al. 1980). Therefore, an evaluation and understanding of the drainages and subsequent effects from hollow fills on low order streams is essential to the preservation aquatic biological communities and water quality in mining regions.

The influence of mining on water quality and benthic macroinvertebrate communities of aquatic systems have been well studied by numerous researchers (Chadwick and Canton, 1984; Clements and Kiffney, 1994; Cherry et al. 2001). Environmental stressors, commonly associated with mining, such as acidification (Courtney and Clements, 1998), metal influx and bioaccumulation (Beltman et al. 1999; May et al. 2001), sedimentation (Wood and Armitage, 1997), and acid mine drainage (Soucek et al. 2001a) have been evaluated for influences upon benthic macroinvertebrate communities. Field studies evaluating active or abandoned mining influences upon stream communities have reported negative impacts to both water quality and biota.

However, little research has focused upon the long-term implications associated with hollow fill drainages constructed from surface mining spoil material.

The focus of this study was to evaluate whether the presence of hollow fill drainages, and associated settling ponds, had detrimental effects on the abiotic and biotic qualities of the receiving streams. Multiple bioassessment techniques and procedures were applied to two streams in southwestern Virginia to evaluate the hollow fill drainages and potential effects. Hollow fill drainages and receiving streams in the upper Powell River watershed and the South Fork of the Pound River (SFPR) were utilized in the study with benthic macroinvertebrate sampling, water column toxicity testing with *Ceriodaphnia dubia*, sediment toxicity testing with *Daphnia magna*, and *in situ* Asian clam (*Corbicula fluminea* [Müller]) toxicity testing used in the assessment. Data were also analyzed to evaluate whether the presence or absence of the settling ponds were functional in dissipating of abiotic parameters and thereby beneficial to the receiving system.

3.2 Methods

3.2.1 Sampling Sites

The hollow fill study evaluated four fills within the headwaters of two different stream systems, the South Fork Pound River (SFPR) and Powell River, located in Appalachian Mountains of Wise County, VA, USA (Fig. 3.1). The two fills evaluated in the SFPR watershed were approximately ½ stream mile apart from one another discharging into the same receiving system (Table 3.1). The uppermost hollow fill was 11 yrs. old (time since completion), with a volume of 420,899 cubic yards with 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. One research site, PdR-D1, was located in the drainage originating from the hollow fill above the two settlings ponds. A site was established downstream of the last settling pond, PdR-D1a, which was the origin of the small tributary. A second site, PdRMix, was established in the small tributary at the confluence with the SFPR in order to assess its influence on the SFPR due to an AMD seep. Therefore, two sites were located in the small tributary; PdR-D1a directly below the settling pond, and PdRMix below the AMD influence. Sites PdR1 and PdR2 were established in the SFPR mainstem above and below the confluence of the tributary. 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. Sites were located above and below the settling pond in the hollow fill drainage, PdR-D2, and the pond drainage, PdR-D2a. Sites within the SFPR mainstem, PdR3 and PdR4, were located above and below the confluence of the settling pond's drainage.

Two hollow fills were assessed in the upper Powell River watershed (Table 3.1). All sites used in the Powell River watershed were located in areas in which the nearby terrain was heavily influenced by surface coal mining. A reference site, PRRF, 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 years 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. Site PR1 was established directly adjacent to the upstream hollow fill. The second hollow fill was 7 yrs. old with a volume

of 400,017 cubic yards, with grass as the dominant vegetation. A study site was established in the hollow fill drainage above the settling pond, PR-D, and in the drainage originating from the settling pond, PR-Da, above the confluence with the receiving system. The final two mainstem sites in the Powell River watershed were established between the two hollow fill influences, PR2, and downstream of the lower hollow fill's influence, PR3. It should be noted that Powell River mainstem sites below the reference including PR1, PR2, and PR3 were receiving potential influences from abandoned deep mining seepages. Small seepage areas located between PR1 and PR3 were evident upon the numerous trips to the watershed.

3.2.2 Water Quality Analysis

Water samples were collected 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 the protocols by the Standard Methods for the Examination of Water and Wastewater (1995). Single measures of water column and sediment metal concentrations were made from water samples and composite sediment samples by the Inductively Coupled Plasma Spectrometry (ICP) lab at Virginia Tech. Water samples were filtered (pore size 0.47 μm) and analyzed for metals including

aluminum (Al), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Sediment samples were digested using (1+1) nitric acid and (1+4) hydrochloric acid under reflux heating according to U.S. EPA protocols (1991) and were then submitted to the ICP laboratory for analysis for total Al, Cu, Fe, Mn, and Zn.

3.2.3 Water Column Toxicity Testing

Water samples were collected in 1-L high density polyethylene bottles upon each visit for toxicity testing upon the cladoceran test species, *C. dubia*. *Ceriodaphnia* were cultured by personnel of the Aquatic Ecotoxicology lab at Virginia Tech in U.S. EPA moderately hard synthetic water (U.S. EPA 1993). Acute toxicity testing had a 0.5 serial dilution of the collected site water. Test organisms, < 24-hrs. old, were used in the dilution series with four replicates, containing five *C. dubia* each, in 50mL beakers. The test occurred under static/nonrenewal conditions for 48-hrs in an incubator at 25±1°C with no feeding regime. Survival was checked and recorded at 24 and 48-hr. intervals and a LC_{50} was generated using LC50/TOXSTAT software (Gulley 1996).

3.2.4 Sediment Toxicity Testing

Sediment samples were collected February and August 2002 for chronic toxicity testing using the cladoceran, *D. magna*, that were raised by personnel of the Aquatic Ecotoxicology lab at VT. *Daphnia* were cultured in 250 mL beakers containing three individuals with filtered Sinking Creek water. The culture water had an average pH, conductivity, alkalinity, and water 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 $CaCO_3$, respectively. Sediment samples were collected using a clean polypropylene scoop per site and placed in Ziploc® freezer bags, and stored at 4 °C for no longer than two weeks. Tests were conducted in accordance to

the procedures of American Society of Testing and Materials (ASTM, 1995) with modifications, using filtered water from Sinking Creek used as the overlying water. Four replications, each containing three *D. magna*, were produced for every research station. 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. Sediment from the reference site within the Powell River was utilized as a control.

3.2.5 *In situ* Asian clam toxicity testing

Asian clams (*C. fluminea* [Müller]), collected from a reference site in the New River near Ripplemead, VA with clam rakes, were returned to the Ecosystem Simulation Laboratory (ESL) at Virginia Tech and stored in a Living Stream® (Frigid Units, Toledo, OH). Clams were then 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 each were placed in polyurethane mesh bags, measuring 18 cm by 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 piece of rebar and placed in riffle habitats at each research sites. Growth and survival were measured every 30 days for an approximate 60-day test period (May 14 thru July 15, i.e. 62 days). Asian clam mortality was assessed as individuals with valves either gapping open or easily teased apart.

3.2.6 Habitat Assessment

Habitat conditions were assessed at each station using the U.S. EPA Rapid Bioassessment Protocols (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 the highest possible score of 200. Therefore, the higher the score a site received, the better the habitat conditions at that particular site. Two individual researchers conducted the habitat assessment separately and the mean scores for each site were recorded and utilized in statistical analyzes. Habitat assessment was important in determining whether landuse or shoreline degradation or postmining conditions were responsible for any impairment within the watershed.

3.2.7 Benthic Macroinvertebrate Survey

Benthic macroinvertebrate surveys were conducted in accordance to the U.S. EPA RBPs (Barbour et al. 1999). A D-frame dipnet, with an 800-μm mesh net, was utilized in conducting qualitative samples per 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 including richness, *Ephemeroptera-Plecoptera-Trichoptera* (EPT) richness, % EPT, a modified % EPT measurement excluding *Hydropsychidae*, %*Ephemeroptera*, and %*Chironomidae.*

3.2.8 Ecotoxicological Rating Procedure

An ecotoxicological rating (ETR) system was developed in order to better evaluate the environmental conditions at the sites within the study (Table 3.2). The ETR system utilized in this study was based on ETR systems implemented in southwestern VA in similar research (Soucek et al. 2000; Cherry et al. 2001). Ecological, toxicological, and physiochemical parameters were selected for analysis by the particular parameters' relevance to mine drainages in aquatic systems. The ecological measurements of total richness, EPT richness, %EPT, %*Ephemeroptera*, and %*Chironomidae* were selected due to the sensitivity or tolerance of the parameter. Transplanted Asian clam (*C. fluminea*) growth was selected as the toxicology measure due to growth sensitivity in the presence of mining toxicants (Grout and Levings 2001; Soucek et al. 2001a). Also, physicochemical parameters, such as conductivity, dissolved Al and Cu concentrations in the water column, and habitat analyses, were selected for relevance to the post-mining conditions within the studied locations.

All parameters, except conductivity, %*Chironomidae* and dissolved Al and Cu, were transformed into a percentage with the highest value measured equaling 1 and subsequent values were then divided by the highest value (value measured at a site/highest value measured in the watershed). Conductivity and %*Chironomidae* measurements were transformed into a percentage with the lowest value measured equaling 1 and subsequent values were then used to divide the lowest value (lowest value measured in the watershed/value measured at a site). Dissolved Al and Cu concentrations for each site were assessed with values under water quality criteria receiving a 1.0 and values over criteria receiving a 0.1. The highest cumulative score a site could obtain

would be 100. The ETR worked on a basis of the higher the total score, the less environmentally impacted the site. Cherry et al. (2001) developed a percentile ranking whereby sites scoring 90% were excellent, 80% were acceptable, 70% were marginal, and ≤ 60% were stressed. The ETR procedure was used against both watersheds combined and for each individual watershed to evaluate the regional conditions.

3.2.9 Statistical Analysis

Data including water quality, sediment toxicity testing, macroinvertebrate indices, Asian clam endpoints, and habitat assessments, were analyzed in the Statistical Analysis System (SAS Institute). Data were tested for normality using the Shapiro-Wilks test (α = 0.05) within SAS. Comparisons of the normally and non-normally disturbed data were made between the sites using the Tukey-Kramer honestly significant difference post-hoc test (α = 0.05) and Wilcoxon Rank sum in SAS. 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® software (Sall and Lehman 1996).

3.3 Results

3.3.1 Water Quality Analysis

Conductivity in the SFPR was significantly higher ($p < 0.05$) in the hollow fill drainages and associated settling pond drainages compared to the receiving stream sites (Table 3.3). The two hollow fills sites, PdR-D1and PdR-D2, had conductivities of 2872 ± 173 μS/cm and 2245 ± 311 μS/cm, respectively, whereas the AMD influence tributary, PdRMix, measured 2654 ± 334 μS/cm. Conductivity of the SFPR receiving stream sites were 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 pH of the SFPR sites ranged from 4.43 ± 0.4 to 8.33 ± 0.3 at PdRMix and PdR1, respectively, with varying levels of significant difference in the watershed. Alkalinity ranged from 18 ± 4 mg/L as $CaCO_3$ and 206 ± 26 mg/L as $CaCO_3$ at PdRMix and PdR1, respectively. Hardness, which was closely related to conductivity ($p = 0.0037$), of the hollow fill and settling pond drainages were significantly higher ($p < 0.05$) than receiving stream sites. Dissolved aluminum and copper concentrations were above the U.S. EPA's nationally recommended WQC (1999) at multiple sites in the SFPR (Table 3.4). Four sites, PdRMix, PdR3, PdR4, and PdR-D2, were above the U.S. EPA chronic criteria for aluminum (0.087 mg/L) with PdRMix having the highest concentration of 36.62 mg/L. Additionally, five sites, PdRMix, PdR2, PdR3, PdR-D2, and PdR4 were above the U.S. EPA's WQC for copper (0.009 mg/L) with PdRMix having the highest concentration of 0.028 mg/L.

Conductivity in the Powell River watershed was highest in the hollow fill drainage, PR-D, with 1748 ± 207 μS/cm, which was significant different ($p < 0.05$) from all other sites that ranged from 896 ± 158 μS/cm to 985 ± 104 μS/cm at PR3 and PR-Da, respectively (Table 3.3). The pH level in the hollow fill drainage, PR-D, was significantly lower ($p < 0.05$) than the upstream reference site, PRRF, yet values only ranged from 7.05 ± 0.4 to 7.76 ± 0.3, respectively. PR-D had the lowest alkalinity and highest hardness for the study sites in the Powell River watershed with 50 ± 9 mg/L as $CaCO_3$ and 1050 ± 144 mg/L as $CaCO_3$, respectively. Metal concentrations in the sediments and water column, which were not above the U.S. EPA's WQC (1999), were

elevated within the hollow fill and settling pond drainages compared to the Powell River receiving stream sites (Table 3.4).

3.3.2 Water Column Toxicity Testing

The most toxic site to *Ceriodaphnia* in the two watersheds was the AMD influenced tributary, PdRMix, with an average LC_{50} of 20.34 ± 4.0% (Table 3.5). The lower hollow fill drainage within the SFPR watershed, PdR-D2, had an average LC_{50} of 60.45 ± 23.2%. The hollow fill drainage within the upper Powell River watershed, PR-D, had an average LC_{50} of 49.88 ± 13.1%. Water samples from the remaining sites within both the SFPR and Powell River watersheds did not report any significant toxicity to *Ceriodaphnia*.

3.3.3 Sediment Toxicity Testing

The first round of sediment toxicity testing involving *D. magna* in February 2002 involved only the receiving stream sites from both watersheds, excluding the hollow fill and settling pond drainages (Table 3.5). There were no significant differences in *Daphnia* survival or fecundity with the highest average neonate production in the SFPR and Powell River sites occurring at PdR1 and PRRF (40.8 ± 3.0 and 43.3 ± 1.8, respectively). The second round of sediment toxicity testing occurred in August 2002 with no significant differences in *Daphnia* survival. Overall, the lowest survival rates for the both watersheds was 33.3 ± 16.9% and 58.3 ± 18% for PdR1 and PR3, respectively. PdR-D2a had the highest mean fecundity within the SFPR watershed of 45.08 ± 8.6 neonates. The lowest mean fecundity rates were at PdR1, PdR-D1, PdRMix, and PdR2, which were significantly lower ($p < 0.05$) than PdR-D2a, with 10.67 ± 13.4, 20.67 ± 12.0, 16.5 ± 6.9, and 18.25 ± 9.6, respectively. There were no significant differences in

fecundity in the Powell River watershed sites. In the SFPR, *Daphnia* survival for the SFPR was negatively related to sediment iron concentrations in the sediments ($p = 0.0009$), and fecundity was negatively related to sediment copper and iron concentrations ($p = 0.0300$ and $p = 0.0250$; respectively). *Daphnia* survival and fecundity in the Powell River watershed were positively related to iron sediment concentrations ($p = 0.0499$ and $p = 0.0411$; respectively).

3.3.4 *In situ* Asian Clam Toxicity Testing

Asian clam survival was significantly reduced ($p < 0.05$) at SFPR sites PdRMix and PdR-D2 with 0.0 ± 0.0% and 20.0 ± 5.8%, respectively (Table 3.5). The best overall clam growth within SFPR was at the settling pond drainages, PdR-D1a and PdR-D2a, with measurements of 2.42 ± 0.6 mm and 1.39 ± 0.3 mm, respectively. *Corbicula* growth in the mainstem SFPR sites, which were significantly lower ($p < 0.05$) than the settling pond drainages, ranged from 0.08 ± 0.03 mm to 0.16 ± 0.03 mm at PdR3 and PdR2, respectively. Water column aluminum and copper concentrations were significantly related to *Corbicula* growth within the SFPR ($p < 0.0010$ and $p = 0.0379$; respectively).

There was no significant mortality in the Powell River receiving stream with the lowest *Corbicula* survival of 90 ± 2.9% occurring at both PRRF and PR3 (Table 3.5). The settling pond drainage, PR-Da, had the best overall growth (0.71 ± 0.26 mm). The lowest clam growth, 0.01 ± 0.02 mm, occurred in the hollow fill drainage, PR-D, which was significantly lower ($p < 0.05$) than all sites except PR2. The upstream reference, PRRF, was significantly different ($p < 0.05$) from the next two downstream sites, PR1 and PR2, with growth of 0.16 ± 0.01 mm, 0.11 ± 0.04 mm, and 0.08 ± 0.03 mm, respectively. *Corbicula* survival exhibited significant positive relationships with

aluminum, iron, and zinc concentrations within the water column of the Powell River watershed sites ($p = 0.0190$, $p = 0.0394$, and $p = 0.0394$; respectively). *Corbicula* survival did exhibit a negative relationship with pH ($p = 0.0080$). No significant relationship could be established between clam growth and metal concentrations.

3.3.5 Habitat Assessment

The poorest habitat conditions in the SFPR and Powell River watersheds were located in the fill drainages (Table 3.3). 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 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. Habitat conditions were positively related to benthic measures of EPT richness ($p = 0.0186$) and %EPT-H ($p = 0.0126$).

3.3.6 Benthic Macroinvertebrate Sampling

Total taxa and EPT richness in the SFPR watershed were highest in sites PdR1 and PdR2, with 9.3 ± 1.0 and 2.8 ± 0.5 taxa, respectively (Table 3.6). The lowest total taxa, 3.3 ± 0.5, and EPT richness, 0.0 ± 0.0, were at PdR-D2 and PdRMix, respectively. Total richness in the SFPR receiving stream decreased downstream where PdR4, with 4.3 ± 1.3 taxa, was significantly lower ($p < 0.05$) than PdR1, with 9.3 ± 1.0 taxa. There were no significant differences between EPT richness among the SFPR receiving stream sites with richness ranging from 1.5 ± 1.0 to 2.8 ± 0.5 at PdR4 and PdR2, respectively. The upper hollow fill drainage, PdR-D1a, had the highest %EPT with 71.9 ± 3.1%. Yet, upon elimination of the more tolerant Trichoptera family, *Hydropsychidae*, the highest %EPT-

H was 13.2 ± 8.2% at PdR1, and PdR-D2a then had a %EPT-H of 0.0 ± 0.0% (Figure 3.2). No ephemeropteran taxa were collected at any site within the SFPR. The tolerant measure of %*Chironomidae* was highest in the lower hollow fill drainage, PdR-D2, comprising 57.5 ± 5.0% of the benthic population which was not significantly different from the SFPR receiving stream sites. Total and EPT richness were negatively related to dissolved iron ($p = 0.0288$) and manganese ($p = 0.0317$) concentrations. EPT richness and %EPT-H were negatively related to conductivity ($p = 0.0228$ and $p = 0.0132$, respectively), whereas %EPT did not have a significant relationship with conductivity ($p = 0.309$).

The Powell River reference site, PRRF, had the highest total and EPT richness of 20.8 ± 1.5 and 10.5 ± 1.0, respectively, both of which were significantly higher ($p < 0.05$) than all other sites (Table 3.6). The fill drainage, PR-D, had the lowest total and EPT richness of 3.0 ± 0.8 and 0.5 ± 1.0, respectively, which were significantly lower ($p < 0.05$) than PRRF. Percent EPT at PR-D was significantly lower ($p < 0.05$) than PRRF, with 7.1 ± 14.3% and 38.2 ± 11.5%, respectively. Additionally, %*Ephemeroptera* was highest at PRRF with 8.9 ± 4.1, whereas no *Ephemeroptera* taxa were collected at either PR-D or PR-Da. Comparisons of the two %EPT measures, %EPT and %EPT-H, exhibited no differences, with neither measure having a significant relationship with conductivity (Figure 3.3). Percent *Chironomidae* at PR-D and PR-Da , 69.3 ± 16.0% and 66.0 ± 5.4%, respectively, was significantly higher ($p < 0.05$) than all other sites, which averaged 21.7 ± 9.6% for the Powell River mainstem sites. Total richness, EPT richness and %EPT were all negatively related to aluminum concentrations in the water column ($p = 0.0458$, $p = 0.0458$, $p = 0.0458$; respectively).

3.3.7 Ecotoxicological Rating

Upon evaluation of both watersheds together, the Powell River reference site, PRRF, was the only site to have a 'passing' ETR score of 85.4 (Table 3.7). According to previous research (Cherry et al. 2000; Soucek et al. 2001), reference sites that normally score above the 80th percentile have no undo environmental stress. The next highest score, 58.5, was the settling pond drainage, PdR-D1a, within the SFPR watershed. The remaining sites within the SFPR ranged from 17.0 to 42.9 at PdRMix and PdR1, respectively. ETR scores for the remaining sites within the Powell River ranged from 30.1 to 54.8 at PR-D and PR1, respectively.

PdR-D1a had the highest ETR, 70.1, upon evaluation of the SFPR separately from the Powell River watershed. In general, all of the ETRs for the SFPR sites increased, yet the values ranged between 63.2 to 22.4 at PdR1 and PdR-D2. PRRF did have the highest ETR with 95.0, when evaluated separately from the SFPR. PR1 and PR2 increased above the 60th percentile, with 61.3.

3.4 Discussion

The evaluation of the hollow fill influences upon the South Fork of the Pound River and Powell River watersheds provided an opportunity to examine the potential alterations to water quality and benthic communities in two distinctly different streams. The water quality of the SFPR receiving stream was not significantly altered. For instance, no significant differences were reported between the mainstem sites, PdR1-PdR4 for any measured water quality parameter. Therefore, the elevated parameters such as conductivity and water hardness were diluted upon entering into the receiving system reporting limited influence. Copper concentrations in the water column, however, were

slightly higher at sites downstream of the AMD tributary compared to the upstream site. Sites in the Powell River watershed had no significant data to suggest the hollow fill drainages were significantly altering the measured water quality parameters. In fact in the Powell River watershed, mean conductivity nominally decreased from 944 μS/cm in upstream reference site to 896 μS/cm in the lowest site. Elevated conductivity is commonly associated with mining influences and drainages (García-Criado et al. 1999; Kennedy et al. 2003). The settling ponds at the base of the fills played an integral role in dissipating metals originating from the hollow fills by either dilution or precipitation, as evident from the lowered levels in the settling pond drainages compared to the fill drainages (Table 3.4). Lastly, there were no patterns in the water quality data of the Powell River watershed to suggest the abandoned deep mine drainages between sites PR1-PR3 were significantly influenced the water chemistry of the stream.

Hollow fill drainages and the AMD influenced tributary, which had elevated metal concentrations, conductivity, and lowered pH, proved to be acutely toxic to *Ceriodaphnia*. PdRMix, the most toxic site with an average LC_{50} of 20.3%, had low pH and dissolved metal concentrations for aluminum and copper that exceeded the U. S. EPA's WQC (1999) measuring 36.62 mg/L (criteria of 0.087 mg/L) and 0.028 mg/L (criteria of 0.009 mg/L) respectively. Soucek et al. (2001b) determined a total aluminum concentration of 2.88 mg/L and Diamond et al. (1997) determined a bioavailable copper concentration of 6.98 μg/L were levels necessary to generate acute toxicity to *C. dubia* at certain water hardness levels. However, the high water hardness for both watersheds would precipitation the majority of the metals bioavailable to the organisms.

The first sediment toxicity test (February 2002) with *D. magna* evaluated only SFPR and Powell River receiving stream sites with no significant *Daphnia* impairment in survival or fecundity. The second test (August 2002) had no significant *Daphnia* impairment for survival or fecundity for the Powell River watershed, or *Daphnia* survival in the SFPR watershed. However, *Daphnia* fecundity rates in the second test for the SFPR did report significant differences among the study sites. Yet, it is important to note there were no significant differences among receiving stream sites, as reported in the first sediment test. The best fecundity rates in the SFPR for the second test were in the settling pond drainages, PdR-D1a and PdR-D2a. The reduced *Daphnia* survival and fecundity in the upstream site PdR1, compared to the pond drainages, were best explained by the elevated iron concentrations within the sediments (Table 4). *Daphnia* survival was negatively related to sediment iron ($p = 0.0009$) and reproduction was negatively related with both sediment copper ($p = 0.0360$) and sediment iron ($p = 0.0250$) concentrations. Sediment toxicity testing by Malueg et al. (1984) significantly related *Daphnia* mortality to copper concentrations within Michigan sediments.

The alterations in fecundity and survival between the two seasons may be best explained by the composition of the sediment itself. The *Daphnia* had no significant mortality in the winter collected sediments from the receiving stream sites with an overall average fecundity for the SFPR and Powell River watersheds of 42.1 ± 2.0 and 39.5 ± 2.4, respectively. The summer collected sediments from the receiving stream sites had varying mortality and overall average fecundity of 21.5 ± 13.5 and 26.6 ± 10.7 for the SFPR and Powell River watersheds, respectively. Thus, there are distinct differences in the results from the two different seasons. Viganò (2000) conducted sediment toxicity

testing with *Ceriodaphnia* with winter and summer collected sediments from the River Po in Italy. Viganò found that the sediments were different in oxygen demands and C/N ratios between the two seasons. Though, there were no data to suggest the constituents of the sediments were significantly different, the fecundity and survival of the *Daphnia* were reduced in the summer compared to the winter, as supported by Viganò's results.

The *in situ* Asian clam toxicity testing provided supportive data for the toxicological results for both water column and sediment testing. *Corbicula* survival was the primary endpoint for the test with significant mortality occurring only in the SFPR watershed at the lower hollow fill drainage, PdR-D2, and the AMD influenced tributary, PdRMix. Water samples collected from each of the sites were found to be acutely toxic to *Ceriodaphnia*. The best *Corbicula* growth in the SFPR occurred in PdR-D1a and PdR-D2a directly downstream of the settling ponds, which may be attributed organic enrichment to due algal growth in the ponds. There was no significant *Corbicula* mortality within the Powell River watershed, as growth was a more significant measure for the two-month study. The low growth measurements within the hollow fill drainage, PR-D, are best explained by limited nutrients and organic content in the drainage, as supported by similar research (Soucek et al. 2001a). The causative factor for the reduced clam growth at sites PR1 and PR2 in the Powell River receiving stream compared to the upstream reference was uncertain. No significant correlations were established between reduced growth values and any abiotic measurement. It is important to note the best *Corbicula* growth for both watersheds occurred downstream of the settling ponds in the presence of higher water column metal concentrations (iron, manganese, and zinc) compared to the receiving stream sites. It is known that filter-feeding organisms, such as

bivalves, can accumulate metals from the dissolved state and in phytoplankton food (Fisher et al. 1996). Yet, metal concentrations would not pose a significant toxicological problem to the clams due to the elevated water hardness. Overall, *Corbicula* growth within the drainages of the settling ponds in both watersheds was not adversely affected by the metal concentration within the water column or phytoplankton community.

Benthic macroinvertebrate data from the SFPR demonstrated a trend of decreased taxa richness associated with the hollow fill drainages and the lowest receiving stream site in the SFPR. Upon evaluation of the more sensitive EPT richness measurement, the entire watershed had decreased richness with only one EPT taxa collected in each hollow fill drainage. Several field studies have demonstrated reduce taxa richness in association with mine drainages (Clement and Kiffney, 1994; Malmqvist and Hoffsten, 1999, Soucek et al. 2000). The %EPT measure was a particular point of interest for the SFPR watershed. Percent EPT had no significant correlation to conductivity within the SFPR watershed, with high %EPT compositions in the settling pond drainages and lowest SFPR receiving stream site. The majority of the taxa comprising the %EPT at these sites were in the family *Hydropsychidae*, which are known to be relatively tolerant to metal uptake (Birge et al. 2000; Wesley et al. 2000). With the exclusion of this tolerant family, %EPT-H reported dramatically different results that correlate with conductivity levels ($p = 0.0111$). The high abundance of *Hydropsychidae* within the settling pond drainages was supported by a significant positive correlation between Asian clam growth and collector filterer populations ($p = 0.0068$). The feeding habits of both the clams and collector-filterers suggest the potential organic enrichment originating from the settling ponds enhanced their populations.

The absence of *Ephemeroptera* taxa from the SFPR watershed suggests some form of stressor(s) negatively influencing the entire watershed. *Ephemeroptera* are well known for their sensitivity to alterations with acidic pH and heavy metal burdens (Hickey and Clements 1998; Courtney and Clements 1998; Malmqvist and Hoffsten 1999). The tolerant measure of %*Chironomidae* was not a viable measurement of hollow fill influences upon the SFPR watershed due to limited significant differences among the sites. The metal accumulation and tolerance aspects of chironomids are well documented (Bervoets et al. 1997; Groenendijk et al. 2000; Bonnet et al. 2001). Again, the entire watershed appeared to be influenced by a stressor as indicated by the absence of *Ephemeroptera*, which would explain *Chironomidae* proliferating where the sensitive species were unable to reside (Richardson and Kiffney 2000). Clements et al. (2000) reported that in the presence of heavy metals, the benthic community shifts from a sensitive to more tolerant community, as supported by the data collected in the SFPR watershed.

The benthic macroinvertebrate data collected from the Powell River watershed had decreased total and EPT richness in the hollow fill drainage, PR-D. However, the hollow fill drainage did have elevated metal concentrations and poor habitat conditions compared to the receiving stream sites. The highest total and EPT richness was recorded in the references, which was significantly higher compared to all other sites in the Powell River watershed. The lower richness at PR1, PR2, and PR3, were not only influenced by hollow fill drainages, but also the abandoned deep mine seepages. The seepages were evident by discoloration of the stream sediment (orange-brownish suggesting iron precipitate). Yet, no water quality data could link the abandoned deep mine drainages to

the lowered richness levels. The %EPT composition suggested decreased sensitive taxa present in the hollow fill and settling pond drainages for all sites the Powell River watershed. Yet, upon elimination of the tolerant trichopteran family, *Hydropsychidae*, no significant differences were generated among the sites with the %EPT-H index due to the absence of *Hydropsychidae* within the drainages. The absence of ephemeropteran taxa from the fill and pond drainages suggested the areas were unsuitable for the proliferation or colonization of sensitive taxa. As stated above, *Ephemeroptera* taxa have been used as biological indicators to metal stressors due to their sensitivity (Hickey and Clements 1998; Courtney and Clements 1998; Malmqvist and Hoffsten 1999). Unlike the SFPR watershed, the benthic data from the Powell River watershed did not suggest a stressor affecting the entire watershed due to the presence of sensitive taxa within the mainstem sites. Thus, the %*Chironomidae* index worked rather well at distinguishing between severely and nominally influenced sites, with the hollow fill and settling pond drainages being significantly higher than the receiving stream sites.

The application of the ETR provided simplified scores to further assess the overall conditions at each study site. The rating worked on the premise that sites scoring below 60 are stressed in some manner, and different from sites scoring >80. When the SFPR was evaluated with the Powell River watershed, the settling pond drainage, PdR-D1a, obtained the highest score with 58.4. PdR-D1a contained high percentage of *Hydropsychidae* and enhanced clam growth, which would suggest organic enrichment. The second highest rating in the SFPR was the uppermost receiving stream site, PdR1, with ratings decreasing downstream. The decrease in ETR scores coincides with a decrease in EPT richness and %EPT-H. The same pattern was reported in the ETR upon

evaluation of just the SFPR watershed, with PdR-D1a and PdR1 scoring 70.1 and 63.2, respectively. The Powell River watershed sites had relatively low ratings as well, with the lowest ratings associated with the hollow fill drainage and settling pond drainage. Comparisons of both watersheds reported the upstream reference site, PRRF, with a rating of 85.4, which suggested no adverse stress upon that particular site. Upon evaluation of merely the Powell River watershed, PRRF scored higher with an ETR of 95.0. Yet, the lower values of the remaining receiving stream sites suggest the sites are negatively influenced by some environmental stressor(s). The decreased richness associated with the lower Powell River sites, compared to the reference, support this theory. However, no significant correlation could be established to explain the decreased richness.

The evaluation of the abiotic and biotic parameters influenced by the hollow fill drainages in the South Fork of the Pound River and the Powell River watersheds provided an opportunity to study two distinctly different systems. The SFPR watershed was significantly stressed by multiple factors, including decreased pH and elevated metal concentrations within the water column and streambed sediment at some of the influenced sites. The absence of ephemeropteran taxa from the watershed was evidence of stressor(s) affecting the entire benthic macroinvertebrate community of the watershed. Conversely, the Powell River watershed study sites, which were not as severely stressed as the SFPR sites, had decreased richness and absence of ephemeropteran taxa associated the hollow fill and settling pond drainages. The application of the ETR reported scores reflecting the poor benthic community and overall conditions of the SFPR watershed. Yet, the ETRs for the Powell River watershed reported poor conditions for the receiving

stream sites, which was not well supported by the benthic and water quality data. The settling ponds at the base of the fills provided a beneficial role in dissipating metal concentrations originating from the fills before draining into the receiving systems. However, the ponds' presence enhanced filtering populations downstream as found by enhanced *Corbicula* growth and macroinvertebrate collector filterer populations. In conclusion, the Powell River and the South Fork of the Pound River watersheds report different results of hollow fill influences upon low order streams. Overall, drainages originating from the fills did not severely hinder the biotic assemblages utilized in the study, suggesting the lowered richness levels and absence of sensitive taxa in the SFPR were linked to environmental stressor(s) influencing the entire watershed. Additionally, benthic macroinvertebrate analysis for the two watersheds showed the efficiency and inadequacy of indices between the two systems. The %*Chironomidae* index worked well in a minimally impacted stream, such as the Powell River watershed, whereas the modified EPT ratio, %EPT-H, worked well in SFPR that was a system influenced by multiple environmental factors.

3.5 References:

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Figure 3.1. A map of the research area in Wise County in southwestern Virginia, USA. Nine research sites were established in the South Fork of the Pound River evaluating two hollow fills and six research sites in the Powell River evaluating two hollow fills.

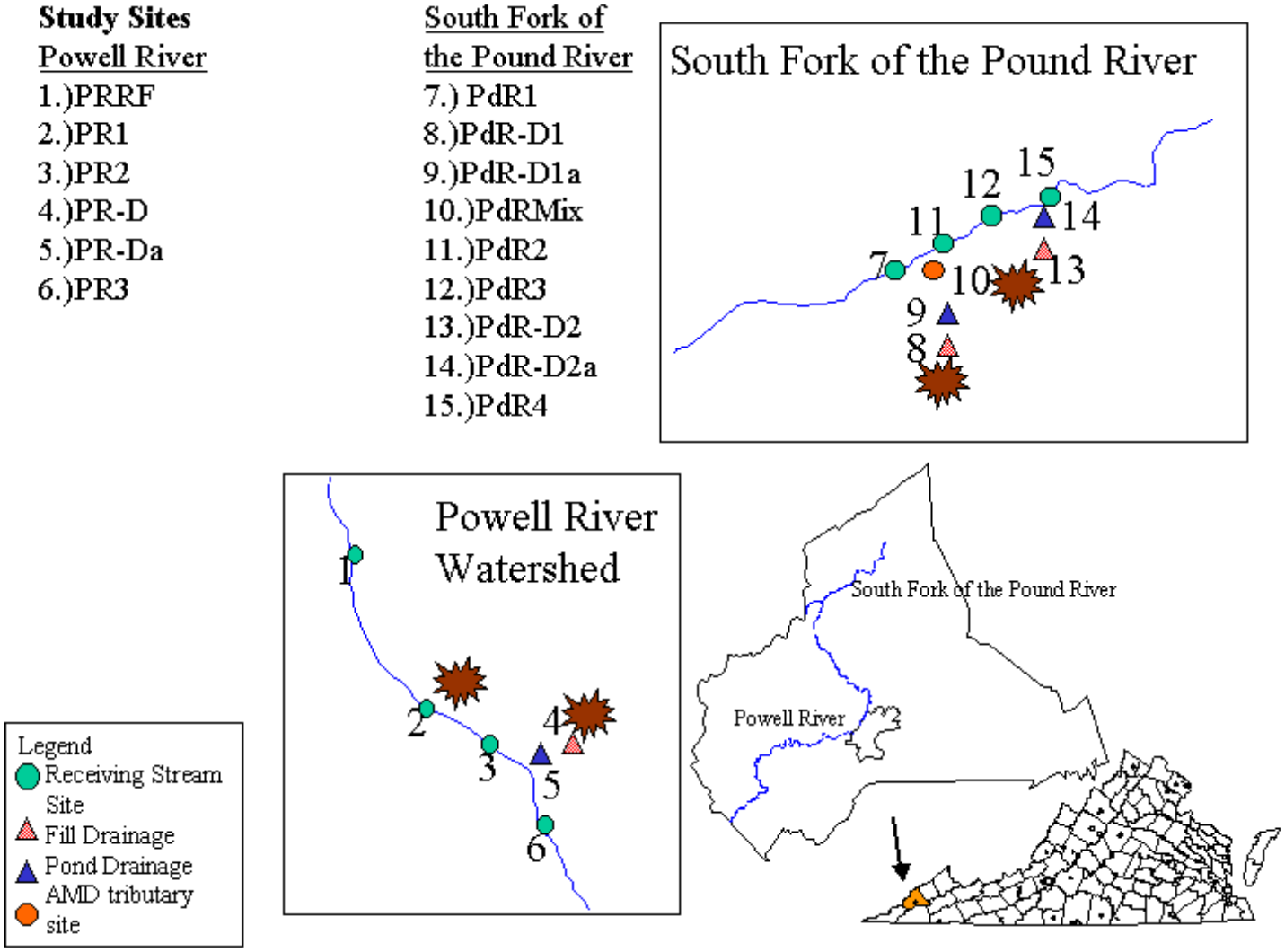

Table 3.1. Summary list of research sites within the South Fork Pound and Powell Rivers within Wise County, VA, USA.

Site Name	Comments
South Fork Pound River Watershed	
PdR1	Upmost site within SFPR, above settling pond drainage PdR-D1a & PdR-Mix
PdR-D1	Drainage originating from a twelve year old hollow fill.
PdR-D1a	Drainage from a settling pond below the PdR-D1 hollow fill drainage. Flows into the SFPR.
PdR-Mix	SFPR tributary receiving PdR-D1a drainage and influence by AMD seeps.
PdR2	SFPR site below the first hollow fill/settling pond drainages and PdR-Mix influences.
PdR3	SFPR site above second hollow fill and settling pond drainages.
PdR-D2	Drainage originating from an eleven year old hollow fill.
PdR-D2a	Drainage from a settling pond below the PdR-D2 hollow fill drainage. Flows into the SFPR.
PdR4	Most downstream site within SFPR, below PdR-D2a.
Powell River Watershed	
PRRF	Reference site within the Powell River watershed.
PR1	Receiving stream site adjacent to a sixteen year old hollow fill.
PR2	Receiving stream site upstream of PR-D and PR-Da drainages.
PR-D	Drainage originating from a seven year old hollow fill.
PR-Da	Drainage from a settling pond below the PR-D hollow fill drainage, flowing into the Powell River watershed.
PR3	Receiving stream site downstream of PR-D and PR-Da drainages.

Table 3.2. List of the chemical, biological, and toxicological parameters used in the ecotoxicological rating (ETR).

Ecotoxicological Rating Procedure	
Parameter	Highest Score
Taxa Richness	12.5
EPT Richness	12.5
% EPT	12.5
% Ephemeroptera	12.5
% Chironomidae	10
Mean Conductivity	10
Water Column Aluminum	7.5
Water Column Copper	7.5
Habitat Assessment	7.5
Mean Clam Growth	7.5
Total	100

Table 3.3. 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
PdRMix	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 3.4. Dissolved water column metal concentrations and streambed sediment concentrations within the Pound and Powell River. Metal concentrations noted with an * denotes values above the U.S. EPA's water quality criteria.

Sites	Al	Fe	Cu	Mn	Zn	Al-Sed	Fe-Sed	Cu-Sed	Mn-Sed	Zn-Sed
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
South Fork Pound River										
PdR1	0.083	0.01	0.004	0.565	0.013	46.64	412.2	0.13	40.24	1.308
PdR-D1	0.008	0.184	0.006	2.797	0.031	48.84	389.5	0.12	41.67	0.559
PdR-D1a	<0.004	0.045	0.007	0.788	0.023	29.73	81.9	0.07	107.8	1.841
PdRMix	36.62*	0.742	0.028*	8.85	0.782	100.3	222.4	0.18	14.62	1.27
PdR2	0.071	0.01	0.009*	1.104	0.021	54.7	246.3	0.12	48.22	1.568
PdR3	0.097*	0.015	0.009*	0.897	0.018	55.8	279.7	0.11	30.95	1.357
PdR-D2	0.787*	0.103	0.021*	6.6	0.205	47.47	258.3	0.12	8.26	0.692
PdR-D2a	0.013	0.192	0.006	2.512	0.092	40.37	119.9	0.08	7.3	0.597
PdR4	0.087*	0.012	0.011*	0.997	0.020	40.86	223.6	0.09	15.02	0.837
Powell River										
PRRF	<0.002	0.03	0.001	0.043	0.008	45.41	203.3	0.12	8.67	1.386
PR1	<0.004	0.176	0.003	0.42	0.009	21.84	99.2	0.03	1.18	0.197
PR2	<0.002	0.021	0.003	0.116	0.007	30.14	145.7	0.05	5.23	0.316
PR-D	0.082	0.404	0.008	21.17	0.317	121.6	105.1	0.22	43.8	1.523
PR-Da	0.011	0.296	0.005	1.033	0.012	41.48	157.7	0.06	27.8	0.574
PR3	<0.002	0.02	0.008	0.18	0.005	22.75	97.6	0.03	5.93	0.233

Table 3.5. Present below is a summary of the toxicological data, including water column, streambed sediment, and *in situ* Asian Clam toxicity testing, for the South Fork Pound and Powell River systems within southwestern VA. Mean values and standard deviations are reported.

	WC Testing	**Sediment Toxicity Testing**				**Sediment Toxicity Testing**				**In situ Asian Clam Test**			
	2002	February 2002				September 2002				2002			
Sites	LC_{50} (%)	Reproduction		Survival (%)		Reproduction		Survival (%)		Growth		Survival (%)	
South Fork Pound River													
PdR1	n/a	40.8 ± 3.0	A	100 ± 0	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
PdRMix	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	40.0 ± 2.1	A	100 ± 0	A	18.3 ± 9.6	CD	66.7 ± 16.9	A	0.16 ± 0.03	C	95 ± 2.5	A
PdR3	N/a	38.4 ± 2.8	A	100 ± 0	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	38.7 ± 2.3	A	100 ± 0	A	30.5 ± 2.8	ABCD	91.7 ± 6	A	0.11 ± 0.02	DE	100 ± 0.0	A
Powell River													
PRRF	n/a	43.3 ± 1.8	A	100 ± 0	A	32.9 ± 5.0	A	100 ± 0	A	0.16 ± 0.01	B	90 ± 2.9	A
PR1	n/a	38.7 ± 2.7	A	100 ± 0	A	22.3 ± 3.8	A	100 ± 0	A	0.11 ± 0.04	C	100 ± 0.0	A
PR2	n/a	42.2 ± 2.2	A	100 ± 0	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	40.9 ± 1.8	A	100 ± 0	A	26.2 ± 19.4	A	58.3 ± 18	A	0.12 ± 0.05	BC	90 ± 2.9	A

Dashed lines represent sites that were not sampled for the particular time period.
Sites with different letters are significantly different from one another.

Table 3.6. Benthic macroinvertebrate data collected for the South Fork of the Pound River and the Powell River in May 2002. Mean values and standard deviations are reported.

Benthic Macroinvertebrate Indices for South Fork Pound River and Powell River, VA, USA.										
South Fork Pound River										
Sites	Richness		EPT Richness		Percent EPT		Percent E		%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
PdRMix	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.

Figure 3.2. Comparisons of the benthic measures of %EPT (Ephemeroptera-Plecoptera-Trichoptera) and %EPT-H (Ephemeroptera-Plecoptera-Trichoptera minus Hydropsychidae) for the South Fork of the Pound River. Percent EPT was not significantly related to conductivity (p = 0.3085), whereas %EPT-H did have a significant relationship (p = 0.0132).

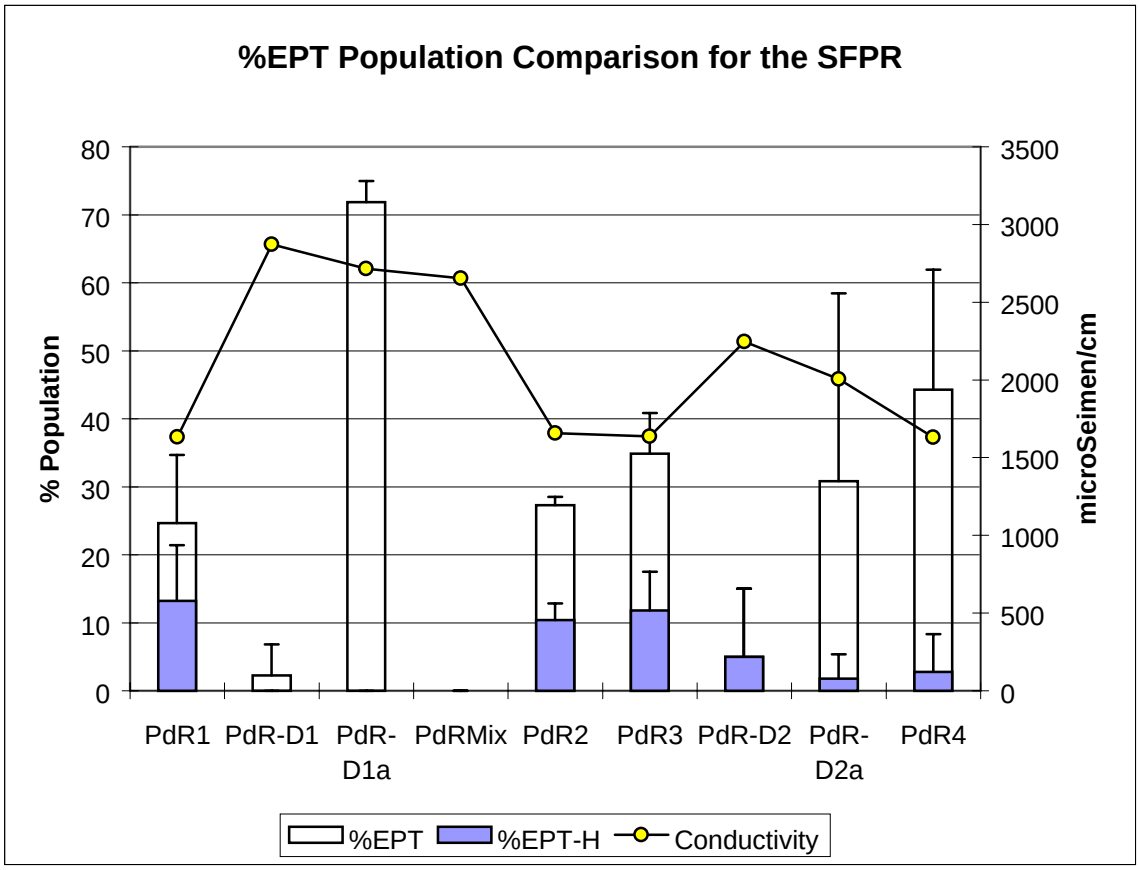

Figure 3.3. Comparisons of the benthic measures of %EPT (Ephemeroptera-Plecoptera-Trichoptera) and %EPT-H (Ephemeroptera-Plecoptera-Trichoptera minus Hydropsychidae) for the Powell River watershed. Neither %EPT or %EPT-H had a significantly relationship with conductivity in the Powell River (p = 0.3287 and p = 0.1562 respectively).

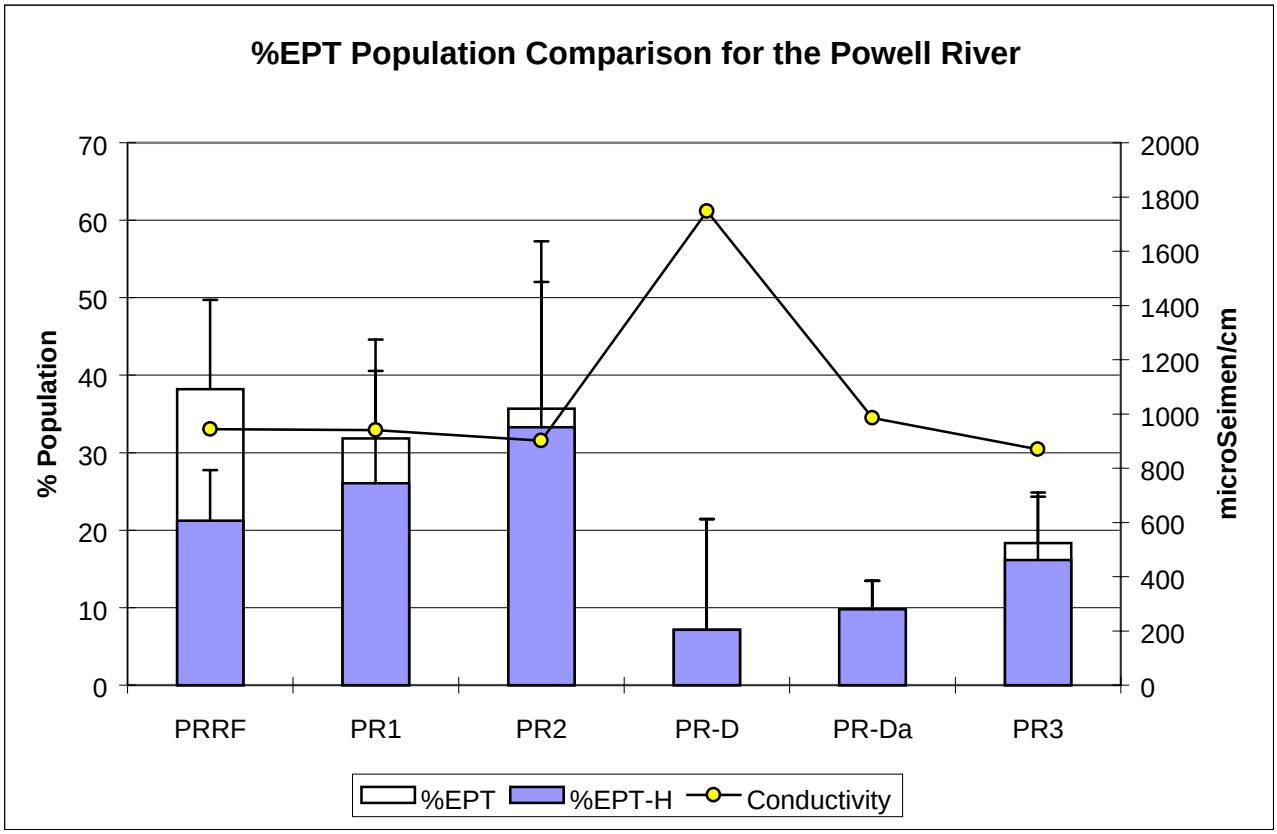

Table 3.7. Ecotoxicological rating scores for the research sites within the South Fork Pound River and upper Powell River watershed. ETRs were scored for both watersheds (A) and each watershed separately (B). Sites score above 80 are considered nominal and sites score below 60 are considered impaired.

Sites	**ETRS**	
	A	**B**
South Fork Pound River Sites		
PdR1	42.9	63.2
PdR-D1	29.2	36.6
PdR-D1a	58.4	70.1
TipMix	17.0	23.4
PdR2	35.2	55.1
PdR3	30.6	49.4
PdR-D2	15.5	22.4
PdR-D2a	42.2	53.3
PdR4	29.1	42.3
Powell River Sites		
PRRF	85.4	95.0
PR1	54.8	61.3
PR2	54.0	61.3
PR-D	30.1	31.6
PR-Da	42.1	49.5
PR3	49.7	54.2

4 Ecotoxicological evaluation of hollow fill maturity and associated settling pond functionality upon low order streams in Virginia and West Virginia.

Abstract. Hollow fills in five different watersheds in Virginia and West Virginia were evaluated assessing drainages originating from the fills and sites below the settling ponds at the base of the fills. Bioassessment techniques, including water/sediment chemistry, acute water column toxicity testing with *Ceriodaphnia dubia*, chronic sediment toxicity testing with *Daphnia magna*, *in situ* Asian clam (*Corbicula fluminea* [Müller]) toxicity testing, and benthic macroinvertebrate surveys, were utilized in evaluating the differences between the fill drainage and sites below the settling ponds. Limited significant differences were reported for water quality; however, there was a consistent trend among all watersheds of higher conductivity and water hardness in the fill drainages compared to sites below the ponds. Water column metal concentrations were lower below the ponds compared to the fill drainages. There were no significant differences in the benthic macroinvertebrate data for each individual watershed, although consistent trends of lower richness, %EPT and elevated *Chironomidae* populations were in the fill drainages compared to the sites below the ponds. These trends were supported by significant relationships in paired t-test analysis for all watersheds. The most significant difference with fill drainages and sites below ponds between watersheds included Asian clam growth and collector-filterer populations, which were both significantly elevated below the ponds compared to the fill drainages. However, the elevated growth and collector-filterer populations were not significantly related to chlorophyll *a* concentrations of the phytoplankton community. Fill maturity was correlated against measured water quality, benthic macroinvertebrate data, and toxicological results to determine if fill age was a factor upon fill drainage influence, but no significant relationship could be established.

4.1 Introduction

Surface coal mining is a major industry in the Appalachian Mountain region of Virginia and West Virginia. In many cases, surface mining is more advantageous than underground mining due to economics, easier access to coal seams, and personnel safety. However, a drawback to surface mining is its subsequent influences and impacts upon the surrounding terrestrial and aquatic environment, including low order streams. Numerous studies evaluating aquatic systems have been conducted in the Appalachian region focusing upon acid mine drainage (Soucek et al. 2000; Cherry et al. 2001), mine reclamation impacts (Matter and Ney, 1981), and industrial discharges (Farris et al. 1998). The recovery of aquatic systems after disturbances, such as surface mining, is important to the preservation of stream habitat and aquatic life. Yet, research that has been conducted upon streams impacts or influences by surface mining have not focused upon the influences of hollow fill drainages, fill maturity, and functionality of settling ponds in dissipating the fill drainages.

Hollow fills are constructed from excess spoil and debris, generated from surface mining, which is not returned to the original mining site upon completion. It is a common practice in the Appalachian region to dispose of the excess materials in nearby or adjacent hollows, which may be the origin of headwater streams, or may drain into low order streams, as in the Appalachian region. Settling ponds are constructed at the toe or base of the hollow fills as a means of passive control to impede the fill drainages from flowing directly into the receiving streams. The settling ponds play an integral role in sediment/metal precipitation of the drainage water before discharging into the stream, which have been considered beneficial buffer zones (Chadwick and Canton, 1983).

The negative influences of mine drainages are an important issue to the water quality of streams and rivers in the Appalachian region, as with any area disturbed by mining activities. Various researchers have evaluated many different of implications that may be associated with mine drainages including; stream sedimentation and loss of habitat (Bonta, 2000), stream acidification (Courtney and Clements, 1998), elevated conductivity (García-Criado et al. 1999; Kennedy et al. 2003), metal influx and precipitation (Roline, 1988; Clements, 1994; Milan and Farris, 1998; Beltman et al. 1999), as well as acid mine drainage (Kiffney and Clements, 1993; Soucek et al. 2000; Cherry et al. 2001). Typical indicators of the aforementioned stressors to the benthic macroinvertebrate community are decreased richness and loss of sensitive taxa (Roline, 1998; Leland et al. 1989). Due to the severity of some types of mine drainages, such as acid mine drainage, an understanding of recovery and stability over time of the water quality and biota of an aquatic system is essential surface mining permitting and protection of streams and rivers. Studies have found the recovery of streams after severe perturbations, such as mine drainages, if the hindering factor(s), such as metal contamination, are eliminated (Nelson and Roline, 1996; Jeffree et al. 2001; DeNicola and Stapleton, 2002).

Our study focused on seven hollow fills in five different watersheds located in the Appalachian Mountain region of Virginia and West Virginia. Evaluations were conducted on sites in drainages originating directly from hollow fills and sites below settling ponds, which were below the fills. The two areas, fill drainages and below the ponds, were studied to evaluate the functionality and influences of the settling ponds and correlation analysis was conducted to evaluate the relationship of measured parameters to

fill maturity to determine whether negative influences, if present, were related to hollow fill age. Bioassessment procedures used in the evaluation included water/sediment chemistry, chlorophyll *a*, toxicity testing using *Ceriodaphnia dubia* and *Daphnia magna*, benthic macroinvertebrate sampling, and *in situ* Asian clam toxicity testing using *Corbicula fluminea*.

4.2 Methods

4.2.1 Study Sites and Age Classification

Seven hollow fills were evaluated in five different watersheds within the Appalachian region of southern Virginia and West Virginia, USA (Fig. 4.1). Fifteen sampling locations were located n the drainages originating directly from the hollow fills and below the discharge of the associated settling ponds. Two watersheds located in Virginia were sampled evaluating three fills; including the South Fork of the Pound River and the Powell River in Wise County. Three watersheds in West Virginia were sampled evaluating four hollow fill drainages and sites below settling ponds; including Lavender Fork in Boone County, and Five Mile Creek and Trace Fork in Mingo County. Sampling sites and conditions are summarized in Table 4.1.

Two hollow fills were located in the South Fork of the Pound River (SPFR) in Wise County, Virginia. A research site was located in each fill's drainage, above the respective settling ponds, and below them to evaluate their functionality and influence (n=4). The first hollow fill in the SFPR was eleven yrs. old (time since completion and bond release), with a volume of 420,899 cubic yards with vegetation limited to grass and small shrubs. The second hollow fill in the SFPR, which was located approximately ½ mile downstream from the first fill, was twelve yrs. old had a volume of 156,029 cubic

yards with grass as the dominate vegetation. A seven-year old hollow fill in the Powell River, in Wise County Virginia, was also evaluated. The Powell River hollow fill had a volume of 400,017 cubic yards, with grass as the dominant vegetation. All three hollow fill drainages evaluated in the Virginia were relatively short drainages (~100-150 m) with limited riparian vegetation.

Sites within the Five Mile Creek watershed, in Mingo County, West Virginia, were utilized to evaluate a seven-year old hollow fill with a volume of 122,100 cubic yards with grass as the major terrestrial vegetation, including small shrubs and limited trees. The Five Mile Creek fill drainage was approximately 100 m in length, with no significant riparian vegetation. Two branches in the Trace Fork watershed were sampled evaluating two hollow fills; a three-year old fill (1999) with a volume of 317,504 cubic yards with terrestrial vegetation limited to mainly grass and a fifteen-year old fill with a volume of 64,312 cubic yards with well established grass, tree, and shrub vegetation. The hollow fill drainage originating from the younger fill in Trace Fork had a long fill drainage, approximately 250-300 m, with significant riparian vegetation consisting of shrubs and established trees. The second hollow fill drainage in Trace Fork was relatively short, 50-75 m, with no significant riparian vegetation. Lastly, a six-year old fill with a volume of 115,840 cubic yards with grass as the dominant vegetation including established shrubs and small trees, was sampled in the Lavender Fork watershed in Boone County. The two hollow fill drainages originating from the fill in Lavender Fork were relatively long (~200-250 m) with riparian vegetation well established.

4.2.2 Water Column & Sediment Chemistry

Water samples were collected seasonally (winter, spring, summer, fall, n = 5) in 2002 with water quality parameters measured each trip. Conductivity and pH measurements were made under field conditions, and the samples were returned at 4°C where alkalinity and hardness were measured in the laboratory at Virginia Tech, Blacksburg, VA, USA. 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 the protocols by the Standard Methods for the Examination of Water and Wastewater (APHA 1995). Additionally, water and sediment samples were collected on one occasion for metal analysis at the Inductively Coupled Plasma Spectrometry (ICP) lab at Virginia Tech. Water samples were filtered (pore size 0.47 μm) and analyzed for dissolved metal concentrations of aluminum (Al), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Sediment samples were digested using (1+1) nitric acid and (1+4) hydrochloric acid under reflux heating according to U.S. EPA protocols (1991). The digested samples were then submitted to the ICP laboratory for analysis for total metal concentrations of Al, Cu, Fe, Mn, and Zn.

4.2.3 Chlorophyll *a* Analysis

The chlorophyll *a* concentrations of the phytoplankton communities of the fill drainages, settling ponds, and sites below the ponds were measured to evaluate the algal communities within those areas. Chlorophyll *a* concentrations were measured by

collecting 1-L grab samples from each site and pond and filtering the sample (pore size 0.47 μm). The filter paper was ground in a tissue grinder in an acetone solution and clarified by centrifugation. The prepared samples were evaluated using the spectrophotometer method detailed in the Standard Methods for the Examination of Water and Wastewater (APHA 1995).

4.2.4 Water Column Toxicity Testing

Water samples were collected in 1-L high density polyethylene bottles each visit for toxicity testing with the cladoceran test organism, *C. dubia*. *Ceriodaphnia* were cultured by personnel of the Aquatic Ecotoxicology lab at Virginia Tech in U.S. EPA moderately hard synthetic water (U.S. EPA 1993). Acute toxicity testing utilized a 0.5 serial dilution of the collected site water. Test organisms, < 24-hrs. old, were used with four replicates, containing five *C. dubia* each. The test 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 and a LC_{50} was generated using LC50/TOXSTAT software (Gulley 1996).

4.2.5 Sediment Toxicity Testing

Sediment samples were collected in August 2002 for chronic toxicity testing using the cladoceran, *D. magna*. *Daphnia* were cultured at the Aquatic Ecotoxicology lab at Virginia Tech in 250 mL beakers containing three individuals in filtered Sinking Creek water. The culture water had an average pH, conductivity, alkalinity, and water 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 $CaCO_3$, respectively. Sediment samples were collected using a clean polypropylene scoop per site and placed in Ziploc® freezer bags, and stored at 4 °C for no

longer than two weeks. Tests were conducted in accordance with the procedures of 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 produced for each site. The organisms were fed a diet of a 50/50 mixture of *Selenastrum capricornutum* and YCT (yeast, cerophyll, and trout chow). Survival and neonate production were recorded each day of the 10-day test. The overlying water was carefully renewed with aerated reference water gently added followed by the daily feeding regime.

4.2.6 *In situ* Asian clam toxicity testing

Asian clams (*C. fluminea*) were collected from a reference site in the New River near Ripplemead, VA with clam rakes and returned to the Ecosystem Simulation Laboratory (ESL) at Virginia Tech and temporary stored in a Living Stream® (Frigid Units, Toledo, OH). Clams were then 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 each were placed in polyurethane mesh bags, measuring 18 cm by 36 cm with 0.5 cm^2 openings, and transported to the sites. Four replicates (20 clams) were fastened and arranged around a piece of rebar and placed in riffles at each research site. Growth and survival were measured at 30 day intervals for an approximate 60-day test period (May 14 thru July 15, i.e. 62 days). Asian clam mortality was assessed as individuals with valves either gapping open or easily teased apart.

4.2.7 Habitat Assessment

Habitat conditions were assessed at each station using the U.S. EPA Rapid Bioassessment Protocols (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 possible cumulative score of 200. Therefore, the higher the score a site receives, the better the overall habitat conditions at that particular site. Two individual researchers conducted the habitat assessment separately and the mean scores for each site were recorded and utilized in statistical analyzes. Habitat assessment was important in determining whether landuse or shoreline degradation or postmining conditions were responsible for any impairment within the watershed.

4.2.8 Benthic Macroinvertebrate Survey

Benthic macroinvertebrate surveys were conducted in accordance with the U.S. EPA RBPs (Barbour et al. 1999). A D-frame dipnet, with an 800-μm mesh net, was utilized in conducting qualitative samples per site. Four, three-minute samples were conducted in a variety of habitats including 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 richness, *Ephemeroptera-Plecoptera-Trichoptera* (EPT) richness, %EPT, a %EPT measurement excluding *Hydropsychidae*, and population composition measurements of %*Ephemeroptera*, and %*Chironomidae*.

4.2.9 Statistical Analyses

Data were analyzed to evaluate for potential differences between hollow fill drainages, above the settling ponds, and sites downstream of the ponds. Data including water quality measurements, sediment toxicity testing, benthic macroinvertebrate indices, and Asian clam endpoints were analyzed in JMP IN® software (Sall and Lehman 1996). Data were tested for normality using the Shapiro-Wilks test ($\alpha = 0.05$). Normally and non-normally disturbed data were compared using the Tukey-Kramer honestly significant difference post-hoc test ($\alpha = 0.05$) and Wilcoxon Rank sums. 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®. Site data were groups according to its status as a fill drainage site or below a pond site, and analyzed using a paired t-test. Data for the two hollow fill drainages in Lavender Fork were averaged together for this analysis. Lastly, correlation analysis was utilized to evaluate the potential relationship of data versus hollow fill age or maturity.

4.3 Results

4.3.1 Water Column and Sediment Chemistry

Conductivity and water hardness were only significantly different ($p < 0.05$) in the Powell River with the hollow fill drainage, PR-D, having measurements of 1748 ± 169 μS/cm and 1050 ± 118 mg/L as $CaCO_3$, respectively, compared to PR1, below the pond, having measurements of 985 ± 84 μS/cm and 537 ± 33 mg/L as $CaCO_3$, respectively (Table 4.2). Each remaining watershed did have a decrease in conductivity and water hardness when comparing the hollow fill drainages to the sites below the settling ponds, yet the difference was not significant. The highest overall average

conductivity was recorded in one of the two hollow fill drainages in the Lavender Fork watershed, LF-D1, with 3050 ± 883 μS/cm. PdR-DB, a hollow fill drainage in the SFPR, had the lowest average pH, 5.89 ± 0.10, for all of the measured sites. Alkalinity fluctuated between the different systems, with PR-D, in the Powell River, and TF-DB, in Trace Fork, having the lowest and highest mean measurements of 50 ± 7 mg/L as $CaCO_3$ and 352 ± 35 mg/L as $CaCO_3$, respectively. There were limited significant differences between hollow fill drainages and respective sites below the settling ponds for both pH and alkalinity.

Dissolved water column metal concentrations for aluminum was the only measured metal above the U.S. EPA's recommended water quality criteria (WQC) (1999). All sites in West Virginia, excluding FMC1, and a fill drainage in the SFPR, PdR-DB, had aluminum concentrations above the U.S. EPA's criteria of 0.087 mg/L (Table 4.3). The highest aluminum concentration in the water column was in PdR-DB with 0.787 mg/L. The overall pattern of metal concentrations in the water column was higher concentrations in the hollow fill drainages compared to the sites below the respective settling ponds. Many metal concentrations in the sediment of the fill drainages were elevated above sediments collected below the settling ponds, however there was no consistent trend as with the water column metals.

4.3.2 Chlorophyll *a* Analysis

One liter grab samples were collected from research sites in the fill drainages, below the ponds, and in the settling ponds in August 2002. The chlorophyll *a* concentrations for the hollow fill drainages and sites below the ponds were not distinctly different from one another (Table 4.4). The greatest difference was in the SFPR at fill

drainage, PdR-DA, and the site below the pond, PdR-1A, with 2.53 mg/m^3 and 15.36 mg/m^3, respectively. It should be noted that there was a series of two ponds between the hollow fill drainage and site below the pond for this area in the SFPR. In general, the chlorophyll *a* concentrations of the phytoplankton communities in the ponds were elevated well above both the fill drainages and sites below the ponds.

4.3.3 Water Column Toxicity Testing

The most toxic site to *C. dubia* was the hollow fill drainage in the Powell River, PR-D, with an average LC_{50} of 49.9 ± 13.1%(Table 4.5). Additional hollow fill sites that were acutely toxic to *C. dubia* included LF-D1 and LF-D2, in Lavender Fork, with average LC_{50}s of 71.6 ± 0.8%and 70.6 ± 23.1% respectively. Lastly, one of the SFPR hollow fill drainages, PdR-DB, had an average LC_{50} of 60.5 ± 23.2% No site below the settling pond in any watershed reported toxicity to *C. dubia* throughout the sampling period.

4.3.4 Sediment Toxicity Testing

Lavender Fork, in West Virginia, was the only watershed that had significant *D. magna* mortality in one of its two hollow fill drainages compared to the site directly downstream of the settling pond with survival rates of 33 ± 39% and 100 ± 0% at LF-D2 and LF1, respectively (Table 4.5). *Daphnia* fecundity, or reproduction, was significantly different between fill drainages and sites downstream of the ponds in two watersheds, Trace Fork and Lavender Fork, in West Virginia. In Trace Fork, the fill drainage, TF-B, was significantly higher ($p < 0.05$) than the site below the pond, TF-1B, with 62.3 ± 5.0 and 37.5 ± 4.9, respectively. Conversely, one of the two hollow fill drainages in Lavender Fork, LF-D2, had significantly lower ($p < 0.05$) *Daphnia* reproductive rates

compared to the site downstream of the pond, LF1, with 16.8 ± 18.6 and 39.5 ± 2.6, respectively. *Daphnia* survival at sites below the ponds was negatively related to sediment metals of Cu ($p = 0.0413$), Fe ($p = 0.0329$), Mn ($p = 0.0301$), and Zn ($p = 0.0301$). No significant relationships could be established for *Daphnia* survival in the fill drainages, yet *Daphnia* reproduction in the hollow fill drainages was negatively related to sediment manganese concentrations ($p = 0.0280$).

4.3.5 *In situ* Asian clam toxicity testing

Significant Asian clam mortality was reported in two watersheds, Lavender Fork and the South Fork of the Pound River (SFPR). LF1, in Lavender Fork, had an average clam survival of 65 ± 10%, whereas both of the corresponding hollow fill drainages in Lavender Fork had survival rates of 100 ± 0% (Table 4.4). Significant clam mortality in the SFPR occurred in the second hollow fill drainage, PdR-DB, with a survival rate of 20 ± 23%, which was significantly lower ($p < 0.05$) compared to the site below the associated settling pond, PdR-1B, with a survival rate of 100 ± 0%. Clam survival was negatively related to aluminum concentrations in the water column in sites below the ponds ($p = 0.0344$).

Clam growth was significantly higher ($p < 0.05$) downstream of the ponds compared to the hollow fill drainages above the ponds in every watershed (Table 4.4). The highest overall clam growth was reported for FMC1 and PdR-1A with 2.4 ± 0.2 mm and 2.4 ± 0.6 mm, respectively. The clam growth for the hollow fill drainages ranged from 0.0 ± 0.0 mm at both PR-D and PdR-DB and 0.3 ± 0.0 mm at TF-DA, respectively. Clam growth in hollow fill drainages was negatively related to water column metals including Mn ($p = 0.0214$) and Zn ($p = 0.0108$) and sediment metals including Fe (p =

0.0359) and Zn ($p = 0.0214$). No relationships could be established for measured parameters and sites below the ponds. Limited differences were reported for the evaluation of the chlorophyll *a* concentrations of the phytoplankton community of the hollow fill drainages and sites below the ponds (Table 4.4). However, the settling ponds did have elevated chlorophyll *a* concentrations suggesting higher algal communities compared to the fill drainages and sites below the ponds.

4.3.6 Habitat Assessment

Habitat assessment was conducted on one occasion in June 2002 (Table 4.2). In general, hollow fill drainages scored lower compared to the sites below the settling ponds, attributing many of the lower scores to limited riparian vegetation and poor substrate. The greatest difference in habitat conditions of a hollow fill drainage and site below a pond in one individual watershed was in the Powell River with scores of 106.5 to 142.5 in PR-D and PR1, respectively. However, the differences between the fill drainages and sites below the ponds were not consistent for each watershed, with many drainages scoring similar to the sites below the ponds.

4.3.7 Benthic Macroinvertebrate Surveys

Five Mile Creek was the only watershed in which all of the benthic measurements for the hollow fill drainage and site below the settling pond were significantly different ($p < 0.05$) from one another. Total richness, EPT richness, %EPT, %*Ephemeroptera*, and %EPT-H (a modified %EPT measurement minus the trichopteran family *Hydropsychidae*) were all significantly lower in the fill drainage compared to the site below the pond (Table 4.6). Percent *Chironomidae* was significantly higher in the fill drainage compared to the site below the pond with $53.3 \pm 7.5\%$ and $35.6 \pm 9.6\%$ at

FMCD and FMC1, respectively. The remaining watersheds did have a decrease in richness and sensitive benthic measurements as well as an increase in %*Chironomidae* comparing hollow fill drainages to sites below ponds, however these differences were not significant. Total richness and EPT richness in the hollow fill drainages and sites below the ponds had negative relationships with similar metals. In the hollow fill drainages, total richness was negatively related to water column copper ($p = 0.0305$), manganese ($p = 0.0017$) and zinc ($p = 0.0378$) and sediment iron ($p = 0.0075$) and zinc ($p = 0.0075$). Total richness in the sites below the settling ponds was negatively related to water column copper ($p = 0.0362$), manganese ($p = 0.0068$), and zinc ($p = 0.0120$) as well as sediment aluminum ($p = 0.0070$), iron ($p = 0.0005$), and zinc ($p = 0.0234$). EPT richness in sites below the ponds were negatively related to sediment aluminum ($p = 0.0267$) and iron ($p = 0.0015$), whereas EPT richness in the hollow fill drainages established no significant relationship to any measured parameter.

Two forms of measurements were utilized to evaluate the population compositions of *Ephemeroptera*, *Plecoptera*, and *Trichoptera* (%EPT and %EPT-H). Percent EPT was significantly higher ($p < 0.05$) in the sites below ponds compared to hollow fill drainages in four watersheds; Five Mile Creek, Trace Fork, Lavender Fork, and the SPFR (Table 4.6). The remaining watersheds had a similar pattern of lower %EPT in the fill drainages compared to the sites below the ponds; however these values were not significant. The modified version of the %EPT measurement, minus the trichopteran family *Hydropsychidae*, was only significantly different in the hollow fill drainage and lower pond site in Five Mile Creek. Percent EPT had a negative relationship with conductivity ($p = 0.0421$) and water hardness ($p = 0.0317$). Percent

EPT-H had a negative relationship with sediment zinc ($p = 0.0362$). It is important to note that *Ephemeroptera* were only collected in the Five Mile Creek and Trace Fork watersheds.

The only consistent significant difference between the hollow fill drainages and the sites below the settling ponds was the composition of the collector filterers of the benthic macroinvertebrate community. In each watershed, collector filterer populations were significantly higher ($p < 0.05$) below the ponds compared to the hollow fill drainages (Table 4.4). The greatest difference between a fill drainage and site below a pond was in the SFPR between PdR-DA and PdR-1A with 2.3 ± 4.5% and 75.4 ± 2.4%, respectively. Measurements of the chlorophyll *a* levels in the fill drainages and below the ponds were not different; however, the ponds themselves had elevated chlorophyll *a* levels compared to both the fill drainages and the sites below the ponds (Table 4.4).

4.3.7 Paired t-test Analysis of Fill Drainages and Sites Below Ponds

Mean data, including water quality, metal concentrations, benthological, and toxicological, for all fill drainages and sites below settling ponds were compared using paired t-test analysis (Table 4.7). Conductivity and water hardness were significantly higher ($p < 0.05$) in the fill drainages compared to the sites below the settling ponds. Conductivity ranged between 2005 ± 610 µS/cm and 1717 ± 763 µS/cm for the fill drainages and sites below the settling ponds, respectively. The pH levels and habitat conditions were significantly lower ($p < 0.05$) in the fill drainages compared to the sites below the ponds. There were no significant differences concerning water column or sediment metal concentrations. Total richness, EPT richness, and %EPT were all significantly lower ($p < 0.05$) in the fill drainages compared to the sites below the settling

ponds. In contrast, %*Chironomidae* populations were significantly higher ($p < 0.05$) in the fill drainages compared to the sites below the settling ponds. Filterer populations, including benthic macroinvertebrate populations and Asian clam, had significantly greater ($p < 0.05$) populations and better growth in sites below the ponds compared to the fill drainages. There were no significant differences concerning clam survival or *Daphnia* survival or fecundity.

4.3.8 Hollow Fill Maturity Analysis

An analysis was conducted to determine whether the age, or maturity, of the hollow fills was relevant to any measured influence, including water quality data, toxicological results, and benthic macroinvertebrate data. The analysis was conducted evaluating the hollow fill drainages and sites below the ponds separately. There were no significant relationships for any measurement against age for either the hollow fill drainages or sites below the settling ponds (Table 4.8).

4.4 Discussion

The results of this study were similar to previous research in streams influenced by mining activities (Roline, 1988; Clements, 1994). Altered water quality and reduction in benthic macroinvertebrate richness have been verified in numerous studies associated with negative influences of mine drainages, as discussed in this study (Beltman et al. 1999; García-Criado et al. 1999; Kennedy et al. 2003). The biotic community of the hollow fill drainages, which had elevated conductivity and water column metals than sites below the ponds, had a more tolerant biotic community compared to sites below the ponds . The lowered EPT richness and %EPT and elevated *Chironomidae* populations in the fill drainages support the theory of a more tolerant community present compared to

the sites below the ponds. A shift in the tolerance of an overall community has been associated with mining influences in previous research (Leland et al. 1989; Clements, 1994).

Water quality of the hollow fill drainages compared to the sites below their respective settling ponds had few significant differences. In general, conductivity and water hardness were lower in the sites below the ponds compared to hollow fill drainages. The Powell River, in Virginia, was the only watershed with significant differences in conductivity and water hardness when comparing the two different areas, with the site below the pond having lower levels in both cases. Conductivity, which has proven to be an excellent indicator of mining activity (García-Criado et al. 1999; Kennedy et al. 2003), was positively related to water hardness ($p < 0.0001$). The lowest pH was in the SPFR with an average of 5.89 ± 0.10, which is not as acidic as pH levels normally associated with AMD influences (Cherry et al. 2001). Paired t-test analysis reported significant differences in conductivity, hardness, and pH. The differences relate well with the trends reported in the individual watersheds. In addition to analyzing the data in the fill drainages and below the settling ponds, hollow fill maturity was correlated to the water quality data in an attempt to determine if a pattern of influence vs. age existed. However, the correlation analysis did not report any significant relationships of water quality to fill maturity.

Water column aluminum was the only metal above the U.S. EPA's nationally recommended water quality criteria (WQC) (1999). The highest water column aluminum concentration was in a fill drainage, PdR-DB, in the SFPR, in Virginia, which also had the lowest average pH of all sites. Yet, metal concentrations in the water column were

generally elevated in the fill drainages compared to sites below the settling ponds. There were instances in which water column metal concentrations were higher below the ponds compared to the fill drainages; however, this was not a common occurrence (Table 4.3). The decrease in the metal concentrations suggest the presence of the ponds were positive due to fill drainage dilution and possible metal precipitation. Unlike the water column metals, the metal concentrations in the sediments had no distinctive pattern. Indeed, many of the metal concentrations in the hollow fill drainages were higher compared to sites below the settling ponds. However, this trend, or pattern, in the sediment metals was not as consistent as the trend for the water column metals. Metal concentrations and hollow fill maturity were evaluated with no significant relationship established between metals and hollow fill age.

Hollow fill drainages exhibited little toxicity to *Ceriodaphnia,* with only Lavender Fork, SFPR, and Powell River fill drainages acutely toxic. The water column metal concentrations at these particular hollow fill drainages were elevated compared to other hollow fill drainages and the respective sites below the settling ponds. However, the high water hardness associated with the water quality of these fill drainages would precipitate the majority of the metals bioavailable to the organisms.

Sediment toxicity testing did not have any significant data or patterns to suggest hollow fill drainage sediments were more or less toxic to *Daphnia* compared to sediment from sites below the settling ponds. The most *Daphnia* mortality for the test did occur in sediments from fill drainages in Lavender Fork, West Virginia, and the SPFR, in Virginia, which also had the lowest fecundity, or reproductive rates, for all sites. However, there were limited significant differences to suggest the hollow fill drainage

sediments were toxic. The *Daphnia* mortality that was reported was negatively related to sediment copper, iron, manganese, and zinc suggesting sites with elevated metals concentrations would be more toxic.

Transplanted bivalve studies have shown significant results evaluating mine drainages and metal influences upon streams systems within the United States and Canada (Belanger et al. 1990; Grout and Levings, 2001; Soucek et al. 2001). Clam survival has proven to be an excellent indicator of severe influence, such as acid mine drainage (Soucek et al. 2001). However, clam mortality was only significant at two sites, which included a fill drainage in the SFPR, PdR-DB, and the site below the settling pond in the Lavender Fork watershed, LF1. Clam survival was negatively related to water column aluminum levels in sites below the ponds. Although PdR-DB did have the highest aluminum concentration, LF1 did have elevated water column aluminum concentrations compared to other watersheds, yet the aluminum concentration was lower than its respective hollow fill drainages that did not have any clam mortality. Therefore, the higher aluminum concentration and limited morality in the hollow fill drainages in Lavender Fork suggest the aluminum concentrations were not the contributing factor for the mortality at LF1. The most significant pattern evident in the clam study involved growth rates between the fill drainages and sites below the 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. Paired t-test analysis comparing all watersheds also reported clam growth higher below the ponds compared to above them. The enhanced growth below the ponds would suggest some type of organic enrichment originating from the ponds. No significant relationship could

be established between clam growth and measured chlorophyll *a* (p = 0.5040). However, the sites were downstream of the settling ponds, which had elevated chlorophyll *a* concentrations suggesting high algal communities. Therefore, it is natural to assume the clams downstream of the ponds had a more abundant food supply than clams in the fill drainages. Analysis of clam survival and growth to fill maturity did not have any significant relationship to suggest hollow fill age was a factor for clam mortality or growth in this study.

Evaluation of the benthic macroinvertebrate community had few significant differences between the hollow fill drainages and sites below the settling ponds among of the individual watersheds. Yet, several patterns were evident among the different benthic macroinvertebrate indices measured. For instance, both total and EPT richness reported lower values in the hollow fill drainages compared to sites below the settling ponds. Additionally, %EPT was lower in the fill drainages compared to sites below the settling ponds, whereas %*Chironomidae*, which are a tolerant *Diptera* family, were higher in the fill drainages compared to the sites below the settling ponds. Indeed, many of these differences were not significant, however, this was a general pattern that was established in each of the watersheds evaluated. The paired t-test analysis, which compared all watersheds, had significant differences for the benthic macroinvertebrate measurements mentioned above as additional support for the general trends reported in each watershed. Previous research assessing mine drainages and influences confirmed that taxa abundance and richness are inhibited by the negative influences associated with mining (Clements, 1994; Beltman et al. 1999; Soucek et al. 2000). The differences suggest a more tolerant community was present in the hollow fill drainages compared to sites below the settling

ponds. A shift from a sensitive based benthic macroinvertebrate community to a tolerant based community in the presences of stressor(s), such as mine drainages, has been evaluated by similar research (Roline, 1998; Leland et al. 1989; Clements, 1994), as supported by the differences in %EPT and %*Chironomidae*. It is important to note the low populations of *Ephemeroptera* in the watersheds studied. Ephemeropteran taxa were only collected in Five Mile Creek and Trace Fork, both in West Virginia, suggesting both hollow fill drainages and sites below the settling ponds in the other watersheds were unsuitable areas for the more sensitive taxa. *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). An evaluation of hollow fill maturity on the benthic macroinvertebrate data did not report a significant relationship in either the hollow fill drainages or the sites below the settling ponds.

The most significant, or consistent, difference between the benthic macroinvertebrate communities in the hollow fill drainages and sites below the settling ponds were in the collector-filterer populations. The sites below the settling ponds had significantly higher collector-filterers compared to the fill drainages for each watershed evaluated. The higher collector-filterer populations, in addition to the enhanced clam growth, suggest organic enrichment originating from the settling ponds. However, as reported with the clams, no significant relationship ($p = 0.5345$) could be established between chlorophyll *a* measurements of the phytoplankton community and the collector filterer populations below the ponds. Yet, again, it is natural to assume the populations

below the populations had a greater food supply than the fill drainages due to the discharge from the ponds.

The two major objectives of this study were to evaluate differences between hollow fill drainages above settling ponds and sites directly below those respective ponds and determine whether fill maturity was related water quality or benthic macroinvertebrate communities present. The collected data had limited significant differences for both the chemical and biological data, however, patterns were evident among the five different watersheds. Additionally, these patterns were significantly supported when data for all watersheds were compared using paired t-tests. For instance, both conductivity and water hardness were elevated in the fill drainages compared to the sites below the ponds. Water column metals were generally higher in the fill drainages compared to the sites below the ponds, with some outliers. Also, in general, the fill drainages had poorer habitat conditions compared to the sites below the ponds, mainly due to poor substrate and limited riparian vegetation. The biological data reported limited significant differences, with evident trends, again supported by the paired t-test analysis. For instance, both total and EPT richness were higher in the sites below the ponds compared to the hollow fill drainages and %*Chironomidae* populations were elevated in the fill drainages compared to the sites below the ponds. The only consistently significant difference between the hollow fill drainages and sites below the settling ponds was evident in the benthic macroinvertebrate collector-filterer populations and Asian clam growth. Both populations were enhanced due to the presence of the settling ponds. Even though no significant correlation could be established between the growth and collector filterers against chlorophyll *a* concentrations, is it natural to assume

the high algal communities in the ponds themselves were present in the discharges providing a more plentiful food source for sites below the ponds compared to the limited food source for the fill drainages. Lastly, upon evaluation of the data for establishing a relationship of hollow fill influence against fill maturity nothing significant could be established. Therefore, the data collected in this study suggest fill maturity had no influence upon the water quality originating from the hollow fill or the benthic macroinvertebrates present in the fill drainages or below the respective settling ponds.

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Figure 4.1. Study area of southwestern Virginia and West Virginia, USA, evaluating hollow fill in five different watersheds.

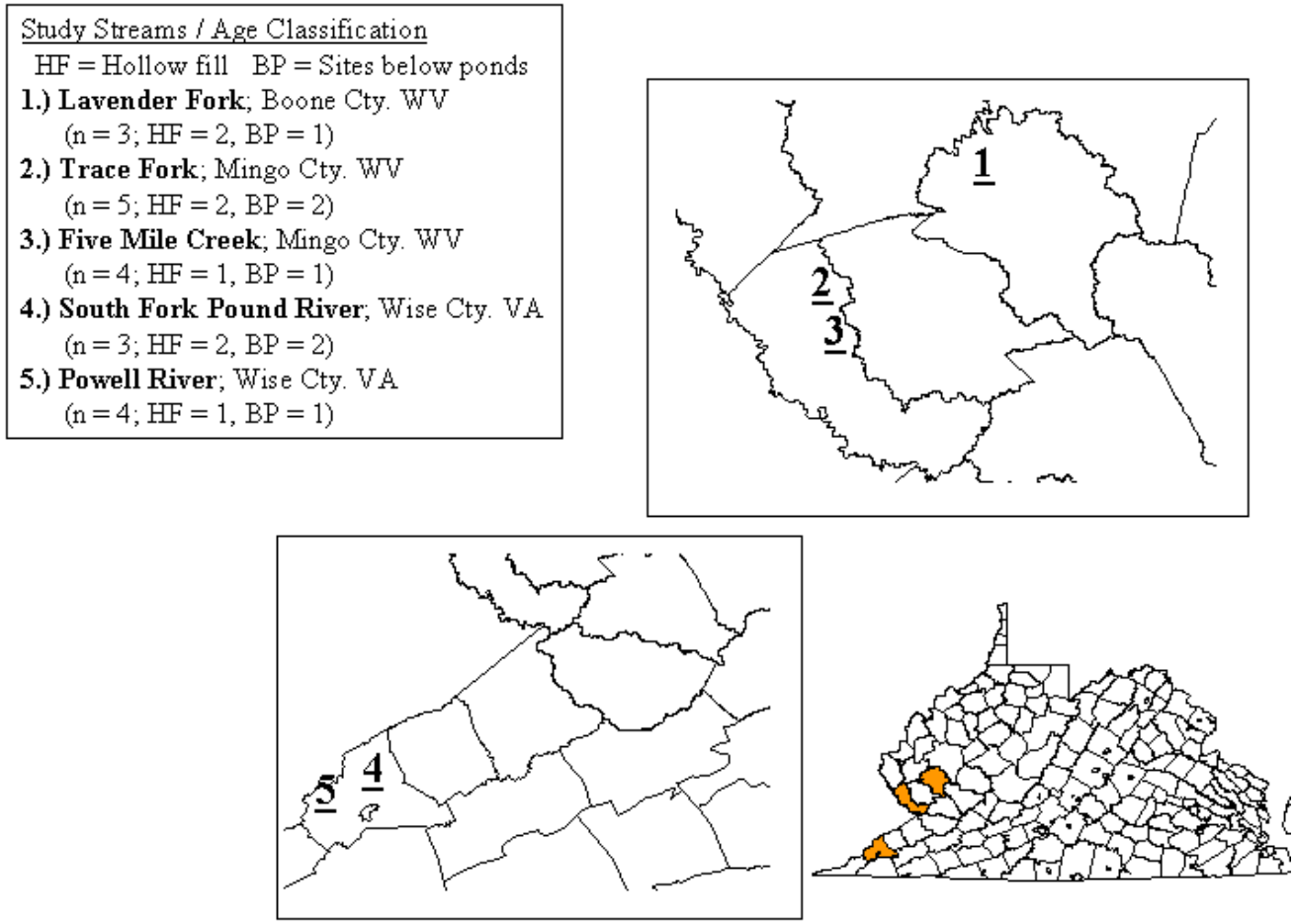

Table 4.1. Location and brief description of sites used in the hollow fill study in Virginia (VA) and West Virginia (WV).

Sites	Location	Comments
Five Miles Creek (WV)		
FMCD	Hollow Fill Drainage	Small drainage from a seven year old hollow fill
FMC1	Below Settling Pond	Site below a settling pond at the base of a seven year old hollow fill
Trace Fork (WV)		
TF-DA	Hollow Fill Drainage	Long drainage from a three year old hollow fill
TF-1A	Below Settling Pond	Site below a settling pond at the base of a three year old hollow fill
TF-DB	Hollow Fill Drainage	Small drainage from a fifteen year old hollow fill
TF-1B	Below Settling Pond	Site below a settling pond at the base of a fifteen year old hollow fill
Lavender Fork (WV)		
LF-D1	Hollow Fill Drainage	Long drainage from a six year old hollow fill
LF-D2	Hollow Fill Drainage	Long drainage from a six year old hollow fill
LF1	Below Settling Pond	Site below a settling pond at the base of a six year old hollow fill
South Fork of the Pound River (VA)		
PdR-DA	Hollow Fill Drainage	Small drainage from an eleven year old hollow fill
PdR-1A	Below Settling Pond	Site below a settling pond at the base of an eleven year old hollow fill
PdR-DB	Hollow Fill Drainage	Small drainage from a twelve year old hollow fill
PdR-1B	Below Settling Pond	Site below a settling pond at the base of a twelve year old hollow fill
Powell River (VA)		
PR-D	Hollow Fill Drainage	Small drainage from a seven year old hollow fill
PR1	Below Settling Pond	Site below a settling pond at the base of a seven year old hollow fill

Table 4.2. Mean water chemistry data (± standard deviation) for sites utilized in the hollow fill study within Virginia and West Virginia.

Water quality data for hollow fill drainages and sites below assoicated setting ponds.												
Sites	**Conductivity**		**pH**		**Alkalinity**		**Hardness**		**Habitat**
	μS/cm				mg/L as $CaCO_3$		mg/L as $CaCO_3$		
Five Mile Creek									
FMCD	1557 ± 545	A	7.42 ± 0.20	B	328 ± 88	A	993 ± 343	A	132.0
FMC1	991 ± 421	A	8.02 ± 0.14	A	200 ± 70	A	552 ± 234	A	138.5
Trace Fork									
TF-DA	1311 ± 324	A	7.84 ± 0.13	B	240 ± 24	A	744 ± 123	A	144.0
TF1A	1248.8 ± 323	A	8.13 ± 0.03	A	222 ± 21	A	700 ± 121	A	146.0
TF-DB	1644 ± 370	A	7.98 ± 0.09	B	352 ± 35	A	1079 ± 183	A	127.5
TF-1B	1356 ± 352	A	8.13 ± 0.06	A	265 ± 37	B	856 ± 204	A	134.0
Lavender Fork									
LF-D1	3050 ± 883	A	8.09 ± 0.03	A	239 ± 23	A	2384 ± 678	A	126.0
LF-D2	2498 ± 780	A	7.92 ± 0.18	A	296 ± 77	A	1882 ± 708	A	118.0
LF1	2720 ± 930	A	8.09 ± 0.05	A	217 ± 62	A	1905 ± 597	A	130.0
SFPR									
PdR-DA	2871 ± 173	A	7.81 ± 0.10	A	103 ± 33	A	2023 ± 117	A	95.5
PdR-1A	2716 ± 159	A	7.76 ± 0.17	A	111 ± 47	A	2017 ± 229	A	104.0
PdR-DB	2132 ± 207	A	5.89 ± 0.10	B	54 ± 47	A	1350 ± 99	A	111.0
PdR-1B	2004 ± 157	A	6.70 ± 0.27	A	32 ± 3	A	1297 ± 121	A	136.0
Powell River									
PR-D	1748 ± 169	A	7.05 ± 0.30	A	50 ± 7	B	1050 ± 118	A	106.5
PR1	985 ± 84	B	7.38 ± 0.09	A	79 ± 10	A	537 ± 33	B	142.5

Sites with different letters are significantly different from one another.

Table 4.3. Metal concentrations from water column and sediment samples of sites utilized in the hollow fill study in Virginia and West Virginia.

Metal Concentrations for hollow fill drainages and sites below settling ponds										
	Dissolved Water Column Concentration					Total Sediment Concentration				
Sites	**Al**	**Fe**	**Cu**	**Mn**	**Zn**	**Al**	**Fe**	**Cu**	**Mn**	**Zn**
	mg/L	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**
Five Mile Creek										
FMCD	0.137 [a]	0.026	0.002	0.092	0.012	8.30	79.80	0.019	1.68	0.203
FMC1	0.079	0.016	0.001	0.007	0.007	8.69	48.60	0.015	2.52	0.164
Trace Fork										
TF-DA	0.129 [a]	0.072	0.004	0.053	0.009	10.61	65.90	0.040	2.25	0.208
TF1A	0.118 [a]	0.055	0.004	0.021	0.009	16.47	66.60	0.048	5.43	0.277
TF-DB	0.147 [a]	0.016	0.003	0.111	0.009	22.83	112.70	0.103	7.86	0.475
TF-1B	0.120 [a]	0.014	0.004	0.006	0.009	18.14	104.80	0.052	7.86	0.365
Lavender Fork										
LF-D1	0.267 [a]	0.015	0.009	0.027	0.019	16.99	114.50	0.060	9.73	0.406
LF-D2	0.248 [a]	0.020	0.008	0.321	0.018	13.73	185.00	0.048	82.10	0.320
LF1	0.223 [a]	0.038	0.008	0.030	0.016	19.63	105.30	0.046	16.12	0.299
SFPR										
PdR-DA	0.008	0.184	0.006	2.797	0.031	48.84	389.50	0.120	41.67	0.559
PdR-1A	<0.004	0.045	0.007	0.788	0.023	29.73	81.90	0.070	107.80	1.841
PdR-DB	0.787*	0.103	0.021*	6.600	0.205	47.47	258.30	0.120	8.26	0.692
PdR-1B	0.013	0.192	0.006	2.512	0.092	40.37	119.90	0.080	7.30	0.597
Powell River										
PR-D	0.082	0.404	0.008	21.170	0.317	121.60	105.10	0.220	43.80	1.523
PR1	0.011	0.296	0.005	1.033	0.012	41.48	157.70	0.060	27.80	0.574

Table 4.4. Evaluation of the influence of the settling pond and natural organic enrichment upon Asian clam growth and benthic macroinvertebrates. Mean collector-filterer populations and Asian clam endpoints (± standard deviation) are reported.

Evaluation of organic enrichment from the settling ponds.							
	Chlorophyll *a* mg/m^3	Collector-Filterers		Asian clam toxicity testing Survival (%)		Growth (mm)	
Five Mile Creek							
FMCD	3.51	3.2 ± 3.2	B	100 ± 0	A	0.2 ± 0.0	B
Pond	11.25						
FMC1	2.28	26.4 ± 7.4	A	95 ± 10	A	2.4 ± 0.2	A
Trace Fork							
TF-DA	1.87	7.2 ± 10.0	B	95 ± 10	A	0.3 ± 0.0	B
Pond	4.78						
TF1A	1.70	50.7 ± 4.5	A	100 ± 0	A	1.3 ± 0.2	A
TF-DB	1.39	2.7 ± 3.1	B	100 ± 0	A	0.2 ± 0.0	B
Pond	12.92						
TF-1B	1.03	17.2 ± 7.2	A	95 ± 10	A	2.0 ± 0.1	A
Lavender Fork							
LF-D1	1.04	24.4 ± 3.4	B	100 ± 0	A	0.2 ± 0.0	B
LF-D2	1.55	2.3 ± 1.6	C	100 ± 0	A	0.2 ± 0.0	B
Pond	8.45						
LF1	1.55	51.4 ± 7.9	A	65 ± 10	B	0.8 ± 0.1	A
SFPR							
PdR-DA	2.53	2.3 ± 4.5	B	100 ± 0	A	0.2 ± 0.0	B
Pond	12.53						
PdR-1A	15.36	75.4 ± 2.4	A	95 ± 10	A	2.4 ± 0.6	A
PdR-DB	1.33	0.0 ± 0.0	B	20 ± 23	B	0.0 ± 0.0	B
Pond	4.96						
PdR-1B	1.34	29.0 ± 24.2	A	100 ± 0	A	1.4 ± 0.3	A
Powell River							
PR-D	2.47	0.0 ± 0.0	B	100 ± 0	A	0.0 ± 0.0	B
Pond	2.95						
PR1	1.77	16.3 ± 4.9	A	100 ± 0	A	0.7 ± 0.3	A

Sites with different letters are significantly different from one another.

Table 4.5. Summary of the mean (± standard deviation) laboratory toxicological testing results involving *Ceriodaphnia dubia* and *Daphnia magna.*

Toxicological data for hollow fill drainages and sites below associated settling ponds.					
	C. dubia	***Daphnia magna* sediment testing**			
		Survival (%)		Reproduction	
Five Mile Creek					
FMCD	n/a	83 ± 33	A	34.9 ± 13.4	A
FMC1	n/a	75 ± 32	A	23.6 ± 8.8	A
Trace Fork					
TF-DA	n/a	92 ± 17	A	41.4 ± 8.0	A
TF1A	n/a	83 ± 33	A	36.9 ± 11.8	A
TF-DB	n/a	100 ± 0	A	62.3 ± 5.0	A
TF-1B	n/a	100 ± 0	A	37.5 ± 4.9	B
Lavender Fork					
LF-D1	71.63 ± 0.80	92 ± 17	A	36.9 ± 1.8	AB
LF-D2	70.63 ± 23.1	33 ± 39	B	16.8 ± 18.6	B
LF1	n/a	100 ± 0	A	39.5 ± 2.6	A
SFPR					
PdR-DA	n/a	50 ± 58	A	20.7 ± 12.0	A
PdR-1A	n/a	100 ± 0	A	34.3 ± 3.8	A
PdR-DB	60.45 ± 23.2	92 ± 17	A	34.5 ± 1.0	A
PdR-1B	n/a	100 ± 0	A	45.1 ± 8.6	A
Powell River					
PR-D	49.88 ± 13.1	100 ± 0	A	30.2 ± 0.8	A
PR1	n/a	100 ± 0	A	32.1 ± 2.5	A

Sites with different letters are significantly different from one another.

Table 4.6. Mean benthic macroinvertebrate data (± standard deviation) collected from the sites utilized in the hollow fill study in Virginia and West Virginia.

Benthic macroinvertebrate community data for hollow fill drainages and sites below assoicated settling ponds.												
	Richness		EPT Richness		% EPT		%Ephemeroptera		%EPT-H		%Chironomidae	
Five Mile Creek												
FMCD	11.8 ± 2.2	B	2.0 ± 0.8	B	5.8 ± 3.0	B	0.6 ± 1.3	B	5.1 ± 2.3	B	53.3 ± 7.5	A
FMC1	19.5 ± 2.1	A	6.8 ± 1.5	A	17.4 ± 3.1	A	6.1 ± 2.1	A	12.5 ± 2.2	A	35.6 ± 9.6	B
Trace Fork												
TF-DA	10.0 ± 2.8	A	1.0 ± 1.4	A	5.7 ± 9.1	B	0.0 ± 0.0	A	1.1 ± 2.1	A	32.0 ± 15.1	A
TF1A	10.3 ± 2.2	A	2.8 ± 1.5	A	31.7 ± 6.3	A	2.0 ± 1.8	A	3.4 ± 3.4	A	18.5 ± 1.2	A
TF-DB	10.0 ± 2.7	A	1.8 ± 1.7	A	19.1 ± 22.2	A	0.0 ± 0.0	A	16.5 ± 19.3	A	41.5 ± 19.6	A
TF-1B	13.3 ± 1.5	A	3.3 ± 1.0	A	19.5 ± 6.6	A	0.0 ± 0.0	A	9.8 ± 4.3	A	29.7 ± 12.1	A
Lavender Fork												
LF-D1	10.5 ± 2.4	A	3.0 ± 0.8	AB	8.5 ± 3.2	B	0.0 ± 0.0	A	6.6 ± 3.5	A	49.3 ± 11.2	A
LF-D2	9.0 ± 1.8	A	2.5 ± 1.0	B	23.8 ± 18.0	AB	0.0 ± 0.0	A	21.5 ± 19.2	A	30.8 ± 14.8	A
LF1	12.3 ± 2.4	A	4.8 ± 1.0	A	32.3 ± 8.3	A	0.0 ± 0.0	A	10.0 ± 4.0	A	35.6 ± 7.7	A
SFPR												
PdR-DA	5.0 ± 1.8	A	0.3 ± 0.5	B	2.3 ± 4.5	B	0.0 ± 0.0	A	0.0 ± 0.0	A	44.9 ± 17.8	A
PdR-1A	7.0 ± 1.8	A	1.0 ± 0.0	A	71.9 ± 3.1	A	0.0 ± 0.0	A	0.0 ± 0.0	A	10.6 ± 3.5	B
PdR-DB	3.3 ± 0.5	A	0.3 ± 0.5	B	5.0 ± 10.0	A	0.0 ± 0.0	A	5.0 ± 10.0	A	57.5 ± 5.0	A
PdR-1B	3.8 ± 1.0	A	1.3 ± 0.5	A	30.8 ± 27.6	A	0.0 ± 0.0	A	1.8 ± 3.6	A	26.3 ± 17.3	B
Powell River												
PR-D	3.0 ± 0.8	B	0.5 ± 1.0	A	7.1 ± 14.3	A	0.0 ± 0.0	A	7.1 ± 14.3	A	69.2 ± 16.0	A
PR1	7.3 ± 1.7	A	1.8 ± 1.0	A	9.8 ± 3.7	A	0.0 ± 0.0	A	9.8 ± 3.7	A	66.0 ± 5.4	A

Sites with different letters are significantly different from one another.

Table 4.7. Results of paired t-test analysis comparing hollow fill drainage (HSD) data to sites below settling pond (SBP) data. Mean values and standard deviations are reported with significance (*p* values) in parenthesis.

Analysis of hollow fill sites and sites below settling ponds using paired t-tests.										
Water Quality Data										
	Conductivity		**pH**		**Alkalinity**		**Hardness**		**Habitat**	
HFD	2005 ± 610	A	7.43 ± 0.76	B	199 ± 128	A	1339 ± 535	A	120 ± 17	B
SBP	1717 ± 763	B	7.75 ± 0.53	A	161 ± 87	A	1123 ± 627	B	132 ± 14	A
	(0.0321)*		(0.0315)*		(0.1127)		(0.0290)*		(0.0308)*	
Water Column Metal Concentrations (Dissolved)										
	Aluminum		**Copper**		**Iron**		**Manganese**		**Zinc**	
HFD	0.22 ± 0.26	A	0.12 ± 0.14	A	0.01 ± 0.01	A	4.43 ± 7.77	A	0.09 ± 0.12	A
SBP	0.08 ± 0.08	A	0.09 ± 0.11	A	0.01 ± 0.00	A	0.63 ± 0.93	A	0.02 ± 0.03	A
	(0.2352)		(0.4461)		(0.2883)		(0.2210)		(0.2033)	
Sediment Metal Concentrations (Total)										
	Aluminum		**Copper**		**Iron**		**Manganese**		**Zinc**	
HFD	39.3 ± 39.9	A	165.9 ± 117.4	A	0.10 ± 0.07	A	21.63 ± 20.91	A	0.57 ± 0.45	A
SBP	24.9 ± 12.5	A	97.8 ± 36.1	A	0.05 ± 0.02	A	24.98 ± 37.50	A	0.58 ± 0.57	A
	(0.2550)		(0.1859)		(0.0875)		(0.7791)		(0.9585)	
Benthic Macroinvertebrate Indices										
	Richness		**EPT Richness**		**%EPT**		**%Ephem.**		**%*Chironomidae***	
HFD	7.6 ± 3.7	B	1.2 ± 1.0	B	8.7 ± 6.3	B	0.1 ± 0.2	A	48.4 ± 12.5	A
SBP	10.5 ± 5.1	A	3.1 ± 2.1	A	30.5 ± 20.2	A	1.2 ± 2.3	A	31.8 ± 17.6	B
	(0.0217)*		(0.0109)*		(0.0490)*		(0.2249)*		(0.0112)*	
Benthological and Toxicological Results										
	Coll. - Filterers		**Clam Growth**		**Clam Sur.**		***Daphnia* Sur.**		***Daphnia* Repo.**	
HFD	4.1 ± 4.7	B	0.2 ± 0.1	B	88 ± 30	A	82.7 ± 19.3	A	35.8 ± 13.4	A
SBP	38.1 ± 21.8	A	1.6 ± 0.7	A	93 ± 13	A	94.0 ± 10.5	A	35.6 ± 6.7	A
	(0.0044)*		(0.0014)*		(0.7220)		(0.2439)		(0.9625)	

Values with different letters are significantly different from one another.
* denotes a significant difference between fill drainages and sites below settling ponds ($p < 0.05$).

Table 4.8. Evaluation of hollow fill maturity upon water quality, toxicological results, and benthic macroinvertebrate data. Listed below are the *p* values correlating age to different parameters ($p < 0.05$ suggest a significant relationship, denoted by an *).

Evaluation of Hollow Fill Maturity on Ecotoxicological Measurements		
	Hollow Fill Drainages	**Below Settling Ponds**
Water Quality	**Age**	**Age**
Conductivity	0.8536	0.6979
pH	0.5258	0.5268
Alkalinity	0.8432	0.7554
Hardness	0.9519	0.6549
Toxicological Data		
Clam Survival	0.9409	0.9670
Clam Growth	0.1712	0.3197
Daphnia Survival	0.4635	0.2594
Daphnia Reproduction	0.3261	0.5203
Benthological Data		
Richness	0.4662	0.6589
EPT Richness	0.5008	0.4835
%EPT	0.6684	0.7546
%Ephemeroptera	0.7382	0.2594
%EPT-H	0.6580	0.7549
%Chironomidae	0.6372	0.6956

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Curriculum Vita

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Education

M.S. student, Biology, Aquatic Toxicology. (Jan. 01 – present)
Virginia Polytechnic Institute and State University. Blacksburg, VA.
B.S. Biology, Radford University. Radford, VA. Cum Laude May 1999.

Organizations and Honors

National Benthological Society
Society of Environmental Toxicology and Chemistry
Phi Kappa Phi National Honor Society (Radford University Chapter)
Kappa Mu Epsilon National Honor Society (Radford University Chapter)
Dean's List, 7 semesters; Radford University

Relevant Work Experience

Graduate Research Assistant. Virginia Tech, Department of Biology. Aquatic Ecotoxicology Lab. Conducted acute and chronic toxicity testing upon industrial effluents and stream sediments, water quality analysis

Graduate Teaching Assistant. Virginia Tech, Department of Biology. Instructed general biology labs sessions. 1/01-12/01.

Quality Control Analyst. C.B. Fleet Pharmaceuticals, Lynchburg VA. Conducted physical inspection of prep assembly products for defects, inspections of manufacturing and productions lines to ensure hygiene, and chemical analysis of pre- and post- product components. 5/99-12/00.

Laboratory Assistant. Radford University. Assisted in laboratory preparation for microbiology and immunology labs. Duties included culturing bacteria stocks, media and buffer prep. 8/97-5/99.

Presentations

Merricks, T. C., D. S. Cherry, C. E. Zipper, and R. J. Currie. Bioassessment of Hollow Fill Drainages on Downstream Biotic Assemblages and Community Structure. Poster. Salt Lake City, Utah. Society of Environmental Toxicology and Chemistry.

Merricks, T. C., D. S. Cherry, C. E. Zipper, and R. J. Currie. Preliminary

Bioassessment of Hollow Fill Drainages on Downstream Biotic Assemblages and Community Structure. Presentation. Wise, VA. Regional Coalfield Water Resource Symposium.

Merricks, T. C., D. S. Cherry, C. E. Zipper, and R. J. Currie. Bioassessment of a Watershed Influenced by Postmining Coal Activites. Poster. Pittsburgh, PA. North American Benthological Society.

Laboratory and Field Skills

Rapid Bioassessment Protocols:
Benthic macroinvertebrate collection and identification
Habitat Assessment
Water Quality Evaluation:
Chemical analysis (pH, Conductivity, DO, Alkalinity, Hardness)
Water and sediment sample preparation for metal analysis
Ceriodaphnia dubia and *Daphnia magna* culturing
Acute and chronic toxicity testing involving *C. dubia* and *Pimephales promelas*
Chronic sediment toxicity testing involving *Daphnia magna*
NPDES permit testing
In situ toxicity testing involving *Corbicula fluminea*

Computer Skills

Microsoft Word, Excel, Frontpage, Powerpoint knowledge
Webpage design and management
Geographical Information System – ArcView 3.1, Spatial Analyst
SAS and JMPIN statistical softwares
Limited trouble-shooting capabilities

Technical Reports

Quarterly Reports to: (Chronic Biomonitoring utilizing *Ceriodaphnia dubia* and *Pimephales promelas*)
International Paper
Celanese Acetate
Brush Wellman
IBM

Special Reports to: (Chronic Sediment Toxicity Testing utilizing *Daphnia magna*)
American Electric Power

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