An Ecotoxicological Assessment of Upper Clinch River Tributaries, Virginia

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ABSTRACT

The Clinch River, Virginia is known for high aquatic biodiversity, particularly Unionidae which are declining at remarkable rates. Studies conducted on the mainstem have only addressed effects of point-source stressors (various toxic spills and effluents from the Clinch River Plant (CRP), Carbo, Virginia) that have been introduced into the Clinch River. It is hypothesized that the tributaries of the Clinch River deliver a variety of stressors to the mainstem, which may affect the diverse fauna. The aquatic health of 19 upper Clinch River tributaries, Virginia, was assessed via ecotoxicological ratings that indicated the least healthy catchments were associated with mining activity (Dumps, Russell and Coal Creeks). Tributaries were categorized by land use and mining streams were significantly different from agricultural and forested streams ($F = 9.63$, $p<0.0001$). Tributaries with ecotoxicological ratings (ETR) <80 from 100 were deemed suboptimal and thus studied further. Using identical response variables and upstream and downstream sites, resulting ETRs for nine streams indicated no model significance regarding land use, year, or site. Variability within treatments and low sampling sizes contributed to lack of significance, and results indicate that future studies need to be designed incorporating sites with analogous land use stressors. This first assessment of upper Clinch River tributaries indicates the catchments requiring remediation are Dumps, Russell and Coal Creeks, while tributaries requiring extensive evaluations are Big, Lick, Swords, Big Spring, Guest River, Cavitts and Middle Creeks. Tributaries that were deemed healthy (ETRs >80) were Big Stony, Copper, Indian, Stock, Little River and Cove Creeks.

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Introduction

Humans began transforming and controlling riverine systems as early as the late 1700s through deforestation, impoundments, channelization, and dredging with no considerations of endemic species. Although the effects of human destruction to water were realized during this time, little effort was made to minimize those impacts. The Clean Water Act of 1972 and subsequent further amendments have improved water quality, but as the human population continues to increase, the degradation to freshwater systems and the over consumption of natural resources becomes more evident. These manipulations and disregard have had a substantial impact on aquatic systems and their biota. Freshwater fauna (fish and mussels) are declining faster than any other faunal group in North America (Ricciardi and Rasmussen 1999). In particular, *Unionidea,* freshwater mussels, are vanishing at the fastest rate of all terrestrial, marine and freshwater fauna (Williams et al. 1993; Bogan 1996; Turgeon et al. 1998; Ricciardi and Rasmussen 1999). Many reasons for their declines have been established, which include: primarily lack of habitat due to anthropogenic activities, lack of specific fish hosts essential during their lifecycle, historical mussel harvests for the manufacturing of pearl buttons, and exotic species competition, particularly with the zebra mussel. Other reasons that have been documented for diminishing assemblages are siltation from runoff and toxic chemicals (Jones et al. 2001; Diamond et al. 2002; Zimmerman 2003). Recent studies indicate that mussel populations downstream of municipal effluents, which are often high in estrogenic compounds, leads to feminization; populations that are dominated by female organisms (Blaise et al. 2003). Minimal male individuals necessary for fertilization may also be attributing to the vanishing freshwater mussels and are also an indication that humans are altering freshwater systems in many ways.

North America is home to 297 species of freshwater mussels, a third of world's fauna. Nearly 72% of North American mussels are threatened, endangered, or of special concern, 7.1% are extinct, and approximately 23% of the populations are considered stable (Williams et al. 1993). The hotspot for current mussel richness and diversity is located in the Southeastern United States in the Tennessee, Cumberland, and Clinch River drainages (Chaplin et al. 2000). This region has the greatest mussel diversity in the world, although these assemblages are diminishing. The Clinch River begins in Tazewell County, Virginia and runs southwesterly for 563 kilometers (350 miles) until it flows into the Tennessee River, at Watts Bar Reservoir near Kingston, Tennessee. However, only 320 km (200 miles) of the Upper Clinch remain free-flowing (Ahlstedt 1984). Although the Clinch River was at one time home to 55 freshwater mussel species, it is currently believed there are only 43 extant species (Ortmann 1918; Stansbery 1973; The Nature Conservancy Dr. Braven Beaty, Abingdon, VA office, personal communication, January 2005). Further, it is believed many of these assemblages are found in the free-flowing Upper Clinch River where approximately 35-40 species exist (The Nature Conservancy Dr. Braven Beaty, Abingdon, VA office, personal communication, January 2005). While many studies have been conducted in the Clinch River in an attempt to understand these declines, no studies have been carried out in the tributaries. The tributaries are thought to deliver a substantial amount of toxicants and sediments to the mainstem and thus need to be assessed and further monitored in order to gain an enhanced understanding for mussel extirpations.

The Upper Clinch River, Virginia, is part of the Ridge and Valley Province, which is composed of layers of folded sedimentary rock having resulted in valleys separated by ridges (Ahlstedt 1984). Typically the ridges are forested and composed of sandstones and shales, while agriculture and urban lands occur in the valleys dominated by limestone, dolomite and shale formations (US EPA 2002). The limestone rock, rich in calcium and magnesium, can neutralize acidic conditions that often occur in mining dominated areas in this region; this is because in hard water, metals bind with carbonates and become less soluble. This balance maybe offset when coal mining dominates the limestone's counteracting abilities. This geology, as well as precipitation and temperature, is a main factor for this area's freshwater mussel diversity.

This project assesses 19 tributaries of the Upper Clinch River as point source influences to the mainstem. Aquatic health of the selected tributaries was measured based on biological, toxicological and chemical factors. Until now, no data existed for these tributaries. In order to understand freshwater systems and their biota, particularly in this instance, unionids, they should be monitored regularly and over extended periods of time. Further, some entire watersheds should be examined, rather than individual streams. The idea of monitoring whole watersheds began in the early 1900s with the origin of the Tennessee Valley Authority, and recent EPA assessments addressing ecoregions are based on this same approach. If freshwater mussels are extirpated, we will witness one of the greatest mass biotic extinctions of our time. The broader implication of this is that we are destroying our ecosystems and reducing species diversity. In order to understand these processes to properly regulate and manage resources, understanding previous and current conditions is essential. This project is the beginning of this process for the Clinch River tributaries.

Future studies on individual tributaries should be conducted to assess their impact on the Clinch River. It is imperative that these studies incorporate several sampling reaches for each stream to assess the health of the entire catchments as well as within the mainstem.

Although the scope of this study could not include multiple sampling sites for each tributary, it is clearly essential in understanding the aquatic health of entire individual streams. Also, studies incorporating land use effects on individual biota, in particular unionids, should be conducted to further understand the stressors that are most imperative to the mussels' demise. Future studies also need to be designed incorporating sites with analogous land use stressors. While it is necessary to incorporate all tributaries' influences, detailed studies focusing on only the agricultural, mining or anthropogenic factors need to conducted because these stressors have different effects on biota. This study also indicates that particular systems are more variable than others suggesting the influencing stressors may have greater effects on aquatic biota at varying times. This further signifies the need to conduct comprehensive studies on fewer tributaries to acquire a thorough representation of stream health for each respective catchment. This preliminary assessment of major Upper Clinch River tributaries indicates the catchments requiring remediation foremost are Dumps, Russell and Coal Creeks, while tributaries requiring extensive evaluations are Big Creek, Lick Creek, Swords Creek, Big Spring, the Guest River, Cavitts and Middle Creek. Tributaries deemed healthy, defined by ETRs >80, were Big Stony, Copper Creek, Indian Creek, Stock Creek, Little River and Cove Creek.

The two chapters of my M.S. Thesis assess the conditions of major tributaries of the Upper Clinch River, Virginia. Chapter one deals with 19 pre-selected major tributaries of this drainage. Aquatic health was assessed via an Ecotoxicological Rating that incorporated biological, toxicological and chemical parameters. Streams were categorized by land use and differences among groups were assessed. Chapter two is based on the results of the first chapter and addresses upstream and downstream sites of nine tributaries using the same methodology to assess aquatic health.

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CHAPTER 1. Land Use Influences and Ecotoxicological Ratings for Upper Clinch River Tributaries, Virginia

Abstract: The Clinch River system of southwestern Virginia and northeastern Tennessee is among the most diversified aquatic ecosystems of the US, but its fauna are in decline. Unionidae (freshwater mussel) species are a major component of the Clinch's aquatic community, and their decline is well documented. Point-source discharges within the Clinch drainage are few, and primary stressors on the biota originate from non-point tributary sources discharged to the mainstem. Currently, the relative influences of tributaries as stressors on aquatic biota are unclear. We studied 19 major tributaries of the free-flowing upper Clinch River, developed an Ecotoxicological Rating (ETR) utilizing eight parameters, and assessed stream quality among land use categories using multivariate analysis of variance. Biological, toxicological, habitat and chemical variables were measured in each tributary, near it's confluence with the Clinch, to examine combined multiple stressors. Geographic Information System (GIS) software was used to quantify land use within each tributary watershed; all tributary watersheds are predominately forested, but agricultural, mining, and developed land uses (urban, transportation) are also present. ETRs indicated that the tributaries draining mining-influenced watersheds had greater potential adverse impact on the mainstem than those draining agricultural or forested watersheds, due to poor benthic macroinvertebrate scores. ETRs ranged from 44 – 63 for mining influenced tributaries compared to agricultural ETRs of 57 - 86 and forested streams of 64 - 91. Mean ETRs for the mining-influenced tributaries (51) were significantly different than ETRs from agricultural and forested streams (75 and 80, respectively), and developed land uses had no significant relationship with ETRs.

1.1 Introduction

Globally, ten percent of the approximate 300 freshwater mussel species have been declared extinct while nearly half of the species remaining are sufficiently threatened and/or rare to warrant some degrees of protection (USFWS 2002). Recent studies indicate that mussel diversity in the upper Clinch River, Virginia has declined over the last 25 years (Ahlstedt 1984; Dennis 1987; Jones et al. 2001; Zimmerman 2003). The upper Clinch River begins in Tazewell, Virginia and flows for 320 kilometers (200 miles) toward the southwest until it joins the Powell River at Norris Run in Tennessee. This section remains free-flowing and has one of the most diverse mussel assemblages in all of North America (van der Schalie 1938; Neves 1991; Chaplin et al. 2000). At one time, a total of 55 unionid species were documented in the Clinch (Ortmann 1918; Stansbery 1973). It is believed there are presently only 43 extant species in the entire Clinch and 35-40 species in the upper Clinch River (The Nature Conservancy Braven Beaty, personal communication, January 2005).

Previous studies have found stressors are present in the Clinch River basin, and appear to be responsible for the reduction in unionid richness (Ahlstedt 1984; Goudreau et al. 1993; Jones et al. 2001; Diamond et al. 2002; Zimmerman 2003). Stressors cited by these authors include untreated and treated wastewater, chemical spills, acid-mine drainage (AMD), exotic species, impoundments, runoff and sedimentation from agriculture and deforestation, and runoff from abandoned and active mining influences and from urban townships. All of these influences occur from anthropogenic activities. Since few point sources are present in the Clinch system and unionid decline has been well documented, the majority of anthropogenic influences contributing to that decline are commonly assumed to come from non-point sources delivered by the tributaries. Despite documented declines in mussel assemblages in the mainstem Clinch River, the relative contributions of tributaries and major anthropogenic land uses (agriculture, coal mining, development) as potential stressors have not been investigated.

Similar studies utilizing the ETR methodology have been used to show the effects of past mining that continue to degrade water quality (Cherry et al. 2000; Soucek et al. 2000; Schmidt et al. 2002). These studies and others have shown abandoned mine lands, acid-mine drainage, and runoff associated with mines to cause stream impairment (Swift 1982; Cherry et al. 2001). Elevated metal concentrations and low pH associated with acid mine drainage have been linked to reduced fitness in bivalves, *Daphnia magna* and benthic macroinvertebrates, and to mouthpart deformities in chironomids (Milan et al. 1997; Soucek et al. 1999; Schmidt el al. 2002; Swanburg et al. 2002). Agriculture nonpoint source pollution is considered the leading source of water quality degradation in rivers according to the US EPA (2003). The US EPA found sedimentation, most of which comes from agricultural lands, to be the major source of water-quality impairment in the US (US EPA 2003) and the primary physical stressor in mid-Atlantic highland streams (US EPA 2001). Agricultural runoff also contributes nutrients (nitrogen and phosphorous) to stream waters, and elevated levels of these constituents can result in excessive algal biomass and low dissolved oxygen (DO) harmful to aquatic life (Belanger 1991; Dobbs and Welch 2000; US EPA 2000). Excessive nutrients were second only to siltation as the leading cause of impairment in rivers (US EPA 2000). "Straight-pipes" and inadequate household on-site sewage systems also occur in some Clinch tributaries.

Numerous studies have been conducted in the Clinch River in an attempt to understand past and present conditions influencing the decline in unionid assemblages and other aquatic species; however, little research has been conducted in the tributaries. The purpose of this study was to conduct an ecotoxicological assessment of 19 major tributaries of the Clinch River to gain inference on their relative influences on mainstem biota, including its freshwater mussels. An ecotoxicological rating (ETR) system was constructed and utilized to provide an integrate assessment of multiple stressors' influence.

1.2. Materials and Methods

1.2.1. Study Sites

Tributaries selected for study are located in Tazewell, Russell and Scott Counties, Virginia (Figure 1; Table 1). Sampling was conducted within 1.5 km upstream of each tributary's confluence with the Clinch River (except for Big Cedar Creek and Guest River, sampled approximately 5.5 km and 8.0 km upstream from the confluence respectively due to accessibility), and each sampling reach incorporated approximately 135-230 m, depending on stream size and site accessibility.

1.2.2. Water Chemistry Analysis

Water samples were collected as grab samples from the stream center, preserved on ice (4°C), transported to Virginia Tech (VT), and acidified (APHA 1995). Collection, transportation and analysis did not exceed 48 hours. Trace metal analysis was conducted at the VT Soil Testing Laboratory using inductively coupled plasma (ICP) spectrometry (APHA 1995). Ions analyzed included Al, Cu, Mn, Fe, and Zn. Total phosphorous (TP) and nitrates were analyzed at the VT Biological Systems Engineering Water Quality Testing Laboratory using the APHA 1995 protocol. Total dissolved solids (TDS) were analyzed according to APHA Standard Methods (1995).

1.2.3. Benthic Macroinvertebrate Sampling and Habitat Assessment

Qualitative sampling was conducted on May 15-16, 2003 according to Barbour et al. (1999) using the 20-kick method in conjunction with habitat assessment. Four replicate qualitative samples were obtained utilizing 800-µm mesh D-frame nets (Wildco 425-D10) and preserved in 95% ethanol until processed and identified to the lowest practical taxonomic level (usually genus) using standard keys (Merritt and Cummins 1996). The West Virginia Stream Condition Index (SCI) was applied as the macroinvertebrate parameter in the ETR (US EPA 2000). The West Virginia index was used because the Virginia index was a draft report at the time of data analysis, and our study streams were more representative of West Virginia steams than the majority of sampled streams in the Virginia SCI. Habitat assessment was conducted in conjunction with benthic sampling according to the US EPA Rapid Bioassessment Protocols (RBPs) (Barbour et al. 1999).

1.2.4. Toxicological Testing

Sediment tests were conducted according to procedures in the American Society of Testing and Materials (ASTM 1995) and Nebeker et al. (1984) with modifications including an overlying reference water (Sinking Creek, Giles Co. VA) used by the Ecotoxicology Laboratory at VT. Toxicity tests were conducted in 250-mL clean, glass beakers with approximately 75 mL sediment and 150 mL of reference water. Five replicates with three *Daphnia magna* in each replicate were subjected to tributary sediments for 10 days. Overlying water was changed and dissolved oxygen was monitored daily to ensure adequate DO. Following water renewal *Daphnia* were fed a daily diet of *Selenastrum capricornutum* green algae. Endpoint measurements were survivorship and reproduction.

Asian clams (*Corbicula fluminea)* for *in situ* toxicity tests were collected from the New River approximately one mile upstream from Big Falls at McCoy, Virginia. Organisms were maintained in Living Streams® (Frigid Units, Toledo, OH), and fed a daily diet of *Neochloris sp*. before being deployed into the tributaries. Clams were measured from their umbo to their ventral margin with ProMax Fowler NSK ® digital calipers (Fowler Co. Inc., Boston, MA, USA). Only clams between 8.0 mm and 12.0 mm were selected for actual testing organisms. Test organisms were placed in 18 cm by 36 cm polypropylene mesh bags with a mesh size of ~0.5 cm^2. Five separately marked bags with four organisms in each bag were placed in each of the 19 tributaries for 60 days. Bags were secured in pools located downstream from riffle areas to ensure adequate DO supply. When no pool existed, best professional judgment was used to choose comparable habitat. Mesh bags were identified as the experimental unit and a mean size was determined for each bag. Each site was represented by five (i.e., n=5/tributary) growth measurements, each derived from changes in mean clam length within each bag. Survivorship also served as an endpoint. The North Fork Clinch River was used as the reference site to make comparisons because clams in Big Stony, the reference site for other parameters, did not grow.

1.2.5. Ecotoxicological Rating

An ETR was devised utilizing eight parameters with the purpose of ranking the tributaries from least to most favorable for aquatic life. This system included an index of benthic macroinvertebrates (SCI), two toxicological indicators *(in-situ* growth of asian clams and *Daphnia magna* reproduction), four water chemistry parameters (total dissolved solids, aluminum, nitrate, and total P) and habitat assessment. Final ETR scores were based on a 100-point system following Soucek et al. (2000), Cherry et al. (2001), and Schmidt et al.

(2002). Except for Al, all parameter values (SCI, *Daphnia magna* reproduction, clam growth, habitat assessment, TDS, TP and NO_3^--N) were calculated by dividing the tributary value by the reference value (North Fork for clam growth, Stock Creek for TP and Big Stony Creek for other parameters). Aluminum values were broken into representative ranges to present degrees of potential impairment based on US EPA water quality criteria (WQC), and thus values were not divided by the reference value. The transformed parameter values, now a percentage of the reference, were placed into one of five categorical ranges so that each parameter for each tributary received 0, 2.5, 5, 7.5 or 10 points (Table 2). For Al, allotted points were as follows: $\geq$0.0900 = 0, 0.0800-0.0899 = 2.5, 0.0700-0.0799 = 5, 0.0600-0.0699 = 7.5, $\leq$0.0500 = 10. For SCI, allotted points were as follows, 0-49 = 0, 50-59 = 2.5, 60-69 = 5, 70-79 = 7.5, and 80-100 = 10. For all other parameters, allotted points were as follows, 0 = 0, 1-25 = 2.5, 26-50 = 5, 51-75 = 7.5, 76-100 = 10. These scores were then multiplied by the ETR weight for a final parameter score. ETR weights were designed as follows: biological and toxicological indicators represent 60% of the system, (macroinvertebrates 40%, *Daphnia* reproduction in sediment tests 10%, *in situ Corbicula* growth 10%), water chemistry represents 30% (Al, TP, NO_3^--N and TDS each at 7.5%), and habitat assessment represents the remaining 10%. All six parameter scores were summed for a final ETR score.

The SCI, which incorporates six macroinvertebrate indices into one numerical value, was utilized in the ETR to represent the benthic macroinvertebrate communities (US EPA 2000). This index weighs the importance of different groups using counts and pollution tolerance, and was weighted more heavily than other parameters in the ETR. Benthic macroinvertebrates demonstrate greater environmental realism and are indicative of stream health in this system. Further, water chemistry parameters were sampled on one occasion and thus do not represent the temporal variability that is inherent in water-quality observations. For these reasons ETR parameters were not weighed equally to determine stream quality.

Daphnia magna reproduction and *corbicula fluminea* growth were used as toxicological indicators. While five metals were analyzed, only aluminum values were used in the ETR because Cu, Fe, Mn, and Zn were either below wavelength detection limits when analyzed, and/or below any acute and/or chronic toxicity values. TP and NO_3^--N were included to represent potential agricultural influences within the watershed. Although US EPA (2002) reference values for the appropriate ecoregion XI: 67 were considered for differentiating between streams they were not used in the ETR parameter scaling because measured nutrients in most samples were below these values. TDS, which have been shown to cause impairment in benthic assemblages and laboratory bioassays at elevated concentrations (Kennedy et al. 2003), was used in the ETR. TDS sources have been linked to treated wastewater effluents and mining (Goodfellow et al. 2000, Kennedy et al. 2003). TDS and Al are included in the ETR as indicators of mining influence.

1.2.6. Land Use

Geographic Information Systems (GIS) software (ArcView 8.3) and National Land Characterization Data (USGS 2003) were used to analyze and quantify land use within the watershed of each tributary. Five land use categories were used: 1) forest (deciduous, evergreen, mixed), 2) agriculture (orchards, vineyards, pasture, hay, row crops, small grains, fallow), 3) developed (residential, commercial, industrial, transportation, urban/recreation grasses), 4) mining (bare rock, sand, clay, quarries, strip mines, gravel pits), and 5) other (shrubland, grasslands, wetlands, open water). The percentage of each watershed occupied by each land use category was determined, and each tributary was based on major land use influence.

1.2.7. Statistical Analysis

JMPIN® statistical software (Sall and Lehman 1996) was used for analyzing each parameter means. We assessed stream quality using eight measures of biological, toxicological, habitat and chemical factors. This method produced multidimensional response vectors for the quality of each stream. Response vectors for each stream served as independent data points for analysis. We tested for variation in stream quality among land use categories using multivariate analysis of variance (MANOVA) for each response vector (Seber 1984, Johnson and Wichern 1988, Johnson et al. 2003). We used Tukey-Kramer Honestly Significant Difference (HSD) pos-hoc test, which tests differences of many pairs of means for one particular response variable, for mean comparisons among land use categories (α=0.05).

1.3. Results

1.3.1. Land Use

The area of catchments studied totaled 2075 km^2, approximately 70% of the 2900 km^2 Clinch drainage above the Copper Creek confluence. Catchments associated with studied tributaries were dominated by forest (54% - 98%) while agricultural land use varied from 1% - 45% (Table 3). Mining land use varied from <1% -7%, and developed land uses ranged from <1% - 6%. The mining land-use figure is interpreted as an indicator of relative mining influence, as the NLCD data used represent mining activity that was detected from a Landsat analysis during the 1991-1993 time frame (Vogelman et al. 2001), and do not reflect current mining activity.

Land use data for the tributaries was used to classify each as belonging to one of three major categories: primarily forested (F), agricultural (A) or mining (M) influenced (Table 3). Tributaries categorized as agricultural include all for which agricultural land uses exceeded 25% of the watershed area; which are located within the limestone valleys southeast of the mainstem and at the mainstem's headwater. All of the remaining tributaries were located in lands northwest of the mainstem, most of which are steeply sloping and where coal deposits are located. Tributaries draining two areas which are less favorable to mining (Stock, Big Stony, and Cove Creeks south of the Guest River, the drainages of which all include portions of the National Forest; and Indian, Middle, and Big Creeks, which drain an area affected by geologic faulting and containing few mineable coal deposits) were classified as primarily forested; and the remaining tributaries were classified as mining influenced. Both TDS and mining land use data were considered in the decision to categorize Indian, Middle, and Big Creeks as primarily forested. Effects of development (urban, residential, transportation) were investigated by identifying those tributaries with watersheds containing >1% developed land uses, which include several large towns (Tazewell, Lebanon and Coeburn).

1.3.2. Water Chemistry

High TDS values (0.50-0.33 g/L) were found in four tributaries, Russell Creek, Copper Creek, Coal Creek and Dumps Creek, three of which are mining influenced (Table 5). Aluminum was detected above WQC (0.087mg/L) in three tributaries (Dumps, Russell and Swords Creek), all of which are mining influenced. All nitrate samples were below US EPA (2002) reference values for nitrate-nitrite within the Ridge and Valley ecoregion (0.23 mg/L as N); the three tributaries (Big Spring, Big Cedar and South Fork, all agricultural) had the highest nitrate values (0.9 mg/L), two of these streams were also >1% developed**.** Ten of the 19 tributaries (four mining influenced, three agricultural, and three forested; and four developed) had TP levels above the US EPA (2002) ecoregional reference (0.1mg/L), but all were below the ecoregion median.

Mean TDS values in mining influenced streams were significantly greater than in agricultural and forested streams (α=0.05; Table 4). The mining influenced tributaries were found to have the highest mean aluminum concentrations (although not significant), while nitrates were significantly higher in agricultural influenced streams ($p<0.0001$). Tributaries with watersheds containing more than 1% developed land uses had nominally higher nitrate concentrations than other tributaries but differences were not statistically significant. TP concentrations did not exhibit any significant differences among land use categories.

1.3.3. Biological Parameter and Habitat Assessment

Mean SCIs were significantly lower in mining influenced streams compared to land use classified by forest and agriculture (Table 4). SCIs were nominally lower in developed tributaries but differences were not statistically significant. SCI scores were highest in Big Stony, Big Cedar, Little River, Big, and Copper Creeks. Lowest scores were obtained in Coal, Lick, Dumps and Russell Creeks (51, 51, 54, 58 respectively), all of which are mining influenced (Table 6). Habitat scores were nominally highest in the forested streams, as expected, and lowest in the mining-influenced streams, but differences were not statistically significant.

1.3.4. Toxicological Parameters

There were no significant differences among land use categories for the mean reproduction in *Daphnia magna*, although mean reproduction was highest in mining influenced tributaries (Table 4). Reproduction was high (134, 111, 110, 108 neonates) for four sub-watersheds (Big Stony, Copper, Russell, Swords Creeks) and low in Coal, Big, Big Cedar, and Big Spring Creeks (54, 53, 50, 47 respectively) (Table 6).

In Situ Corbicula survivorship was greater than 80% in all tributaries except Big Creek with 55% mortality. While mining influenced tributaries had a greater mean clam growth, there were no significant differences among the three land use categories (Table 4). Clams grew the least Swords, Middle and Cavitts Creeks, with 0.31, 0.42, 0.44 mm respectively (Table 6). Developed tributaries had greater mean clam growth compared to undeveloped tributaries, but were not significantly different. Clams in Coal Creek were not recovered and thus ETR points sum was divided by 0.90 so that the overall ETR score and stream was not penalized.

1.3.5. Ecotoxicological Scores for Selected Clinch River Tributaries

Mean ETRs were significantly lower in mining-influenced (51) than in agricultural (75) and forested (80) tributaries (Figure 2a) and (Table 4). Mean ETRs in forested streams were nominally higher than agricultural stream scores, but not significantly different. Six tributaries received ETRs < 60 (Dumps, Swords, Coal, Russell, Lick and Cavitts Creeks) (Figure 2b). Four tributaries had ETRs in the range of 60-69 (Guest, Middle, South Fork and Big Cedar); while four were in the range of 76-79 (North Fork, Big Spring, Cove and Big Creeks), and five streams had ETRs >80 (Little River, Stock, Copper and Indian, Big Stony Creeks).

1.4. Discussion

Mining influenced tributaries were found to have higher TDS and Al concentrations, as expected. Geologic disturbance through mining commonly increases TDS drainage waters, while acids released by mining can mobilize Al. Only three of the six mining-influenced streams had Al concentrations exceeding the 0.83 mg/L average and may reflect the fact that acid drainages are not common occurrences in the Virginia coalfields, as in Appalachian coal areas located further west. Because acid releases from active mines are controlled by regulation, it is likely that these streams' higher Al concentrations are a result of past mining.

Nitrates were highest in streams draining agricultural watershed, as expected, but TP was highest in streams draining mining watersheds. This latter fact may reflect the fact that we relied upon base-flow sampling, which is not a favorable measurement of agricultural-origin P that tends to be associated with transported sediments. Two of the mining-influenced watersheds (Guest River and Swords Creek) had the highest TP concentrations (>0.18 mg/L). We believe that these TP levels originated from land uses other than mining, such as sewage treatment plants in the Guest, and discharges from households and failing septic systems. Water-quality criteria for nutrients in free-flowing streams have not been established, and nutrient measurements were below ecoregion maximums. Thus, there is no evidence for nutrient impairment in any of these streams.

It is difficult to make distinctions between nutrient levels that augment conditions for aquatic life from those that impair conditions, and enrichment levels that are harmful to aquatic invertebrates have not been established. Several agricultural and mining influenced streams had both high and low response values, reinforcing this difficulty. No standard measures exist for assessing non-point source pollutants introduced to the system by straight pipes, small industry, fuel storage, dumping and/or erosion land uses. Furthermore, such stressors are variable with frequency, durations, components and concentrations. The extent to which influences by such stressors on stream biota is unknown.

The ETR parameter that demonstrated the greatest difference between tributary types was the SCI (Figure 3). Mining-influenced sites scored lowest on the habitat measure, which suggests that degraded habitat may have been one factor that influenced SCI scores. SCI was the most heavily weighted ETR component, and the poor SCI scores at the mining-influenced sites are the primary factor responsible for these sites' low ETR ratings. While these low SCI scores represent pollution tolerate taxa and deficient sensitive taxa, the scores' implications, relative to mining-influenced tributaries' impact on the mainstem biota, is unclear considering the mining-influenced scores for the toxicological variables were comparable among all land use.

Freshwater mussel species are of priority concern in the Clinch. These species are filter-feeders that are closer in physiology to *Corbicula* than benthic macroinvertebrates. Although this fact may lead some researchers to weigh *Corbicula* studies with greater emphasis than macroinvertebrates studies, considerations must be taken regarding the fact that *Corbicula* and unionids reside in different habitats and life stage requirements are exceptionally different for each respective organism. Existing stream benthic macroinvertebrates metrics assess stream quality on a larger scale because they represent a number of different life-stages, habitats and feeding groups compared to *Corbicula* and *Daphnia*. Further, while unionids are native, *Corbicula* are exotic and are thus able to thrive under varying conditions while unionids are most likely more sensitive to shifting conditions within their home range.

Several studies have successfully used Asian clams to detect acid-mine drainage and heavy metal impairment (Belanger et al. 1986; Doherty et al. 1988; Soucek et al. 2001); and sediment tests using *Daphnia magna* have been used for years to detect heavy metal impairment (Soucek et al. 1999; Salomons et al. 1987). However, the use of these organisms in this study were not as effective in detecting mining impairment because these subwatersheds are not acutely toxic and this study addresses multiple stressors influencing each catchment rather than one particular stressor. For these reasons we believe the best determinant for assessing stream quality, in this system, was the benthic macroinvertebrates.

1.5. Conclusions

The results from this study suggest that mining-influenced tributaries have a more negative influence than other tributaries on stream health. ETR differences among $\geq$1% developed and other tributaries were not statistically significant. The major contributor to the mining influenced tributaries' low ETR score was the benthic macroinvertebrate communities, which were seriously degraded in these tributaries compared to streams with watersheds dominated by agriculture and forest cover. However, the implications of mining-influenced tributaries' low ETRs regarding aquatic biota in the mainstem is unclear because, with the exception of TDS, other parameters did not reveal degraded conditions.

1.6. Acknowledgements

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Table 1.1. Abbreviated summaries for Clinch River Tributaries listed from headwaters to bottom of study area. Refer to Figure 1 for tributary location.

#	Tributary	Current Summary
1.	North Fork	Agriculture land use, flows through town of Tazewell. TMDL[a] 7-04.
2.	South Fork	Affected by siltation from highway construction. TMDL[a] 7-04.
3.	Cavitts Creek	Headwaters originates from lake drainage; petroleum and recycling center along stream bank.
4.	Indian Creek	Active and abandoned coal mining in upper reaches of watershed.
5.	Middle Creek	Recovering from reclaimed coal processing site and hollow fill seeps.
6.	Big Creek	Runoff from houses, wastewater treatment plant. Reclaimed mine land in upper reaches.
7.	Coal Creek	Active and abandoned coal mining (four existing permits in upper subwatershed), straightpipe septic systems, railroad track influence.
8.	Swords Creek	Active and abandoned mining, runoff from masonary plant, houses.
9.	Little River	Agricultural/pasture, potential runoff from agriculture and houses.
10.	Big Cedar Creek	Wastewater treatment plant, previously pasture/livestock area.
11.	Dumps Creek	Substantial impairment from long-term active mining and disposal sites. TMDL[a] (TDS/TSS) 7-04.
12.	Big Spring	Straightpipe septic systems.
13.	Lick Creek	Active and abandoned mining.
14.	Russell Creek	Active and abandoned mining. Current (2004) permits exist. Holding pond at toe of hollow fill.
15.	Guest River	Active mining, abandoned mines, AMD and coal storage piles. Flows through coal housing facilities in Norton, VA. TMDL[a] (sediments) 7-04.
16.	Big Stony Creek	Reference stream. Watershed consists of national forest with little to no homes, roads, agriculture.
17.	Cove Creek	Active livestock grazing.
18.	Stock Creek	Closed lithium mine upstream.
19.	Copper Creek	Dominated by agricultural land use.

[a]Total Maximum Daily Loads sources concluded by Department of Environmental Quality.

Table 1.2. Developed ranges with corresponding points used in the ETR.

	Allotted Points in ETR					
	0	**2.5**	**5**	**7.5**	**10**	
Parameter			**Range**			**% of System**
SCI[a]	0-49	50-59	60-69	70-79	80-100	40
DR[b]	0	1-25	26-50	51-75	76-100	10
CG[c]	0	1-25	26-50	51-75	76-100	10
HA[d]	0	1-25	26-50	51-75	76-100	10
TDS[e]	0	1-25	26-50	51-75	76-100	7.5
Al[f]	0.0900-.0999	0.0800-.0899	0.0700-.0799	0.0600-.0699	0.0500-.0599	7.5
TP[gh]	0	1-25	26-50	51-75	76-100	7.5
Nitrate-N[h]	0	1-25	26-50	51-75	76-100	7.5
Total						100

[a]Stream Conditon Index

[b]*Daphnia* reproduction

[c]Clam growth (mm)

[d]Habitat assessment

[e]Total dissolved solids (g/L)

[f]Aluminum (mg/L)

[g]Total phosphorous

[h]mg/L

Table 1.3. Distribution of major land uses within tributary watersheds[a] and each tributary's major land-use influence classification.

Tributary	Percent Forest	of Agriculture	Watershed Developed	Area Mining	Categoryb
4. Indian Creek	89.2	9.5	0.3	0.8	F
5. Middle Creek	97.0	1.2	0.6	1.1	F
6. Big Creek	95.6	1.5	1.2	1.6	F*
16. Big Stony	98.4	1.4	0.0	0.1	F
17. Cove Creek	88.0	11.9	0.0	0.0	F
18. Stock Creek	96.3	3.4	0.1	0.0	F
1. North Fork	54.5	43.2	2.0	0.1	A*
2. South Fork	54.1	44.1	1.6	0.2	A*
3. Cavitts Creek	64.4	33.9	1.1	0.0	A*
9. Little River	54.5	44.6	0.6	0.2	A
10. Big Cedar Creek	71.1	27.9	0.9	0.0	A
12. Big Spring	64.8	32.6	2.5	0.1	A*
19. Copper Creek	72.8	23.7	0.4	0.0	A
7. Coal Creek	87.7	6.9	1.7	3.7	M*
8. Swords Creek	91.4	5.4	0.3	2.7	M
11. Dumps Creek	90.6	1.5	0.5	6.6	M
13. Lick Creek	92.5	4.7	1.2	1.5	M*
14. Russell Creek	88.5	3.8	0.1	7.3	M
15. Guest River	78.9	5.6	5.7	9.0	M*

[a]Percentages do not add up to 100 because lands in other land use categories are not represented.

[b]F = forest, A = agriculture, M = mining subwatersheds. Asterisk (*) denotes developed subwatersheds (>1%).

Table 1.4. Mean parameter estimates $\pm$ SD for response variables used in multivariate analysis of variance (MANOVA) ($F = 9.63$, $p < 0.0001$). The ETR was constructed based on the following: a biological parameter, the Stream Condition Index (SCI); toxicological parameters, *daphnia* reproduction (DR) and clam growth (CG) (mm); chemical parameters, total dissolved solids (TDS) (g/L), aluminum (Al) (mg/L), total phosphorous (TP) (mg/L) and nitrate-N (NO_3^--N) (mg/L); and habitat assessment (HA).

ETR Parameter		**Land Use**	
	Forest (n=6)	**Agriculture (n=7)**	**Mining (n=6)**
SCI	85 $\pm$ 5.6 A	84 $\pm$ 10.9 A	58 $\pm$ 7.7 B
DR	73 $\pm$ 30.9 A	74 $\pm$ 24.9 A	81 $\pm$ 27.0 A
CG	0.725 $\pm$ 0.28 A	0.909 $\pm$ 0.32 A	0.968 $\pm$ 0.41 A
TDS	0.125 $\pm$ 0.061 A	0.193 $\pm$ 0.091 AB	0.322 $\pm$ 0.110 B
Al	0.0683 $\pm$ 0.013 A	0.0668 $\pm$ 0.016 A	0.0934 $\pm$ 0.048 A
TP	0.29 $\pm$ 0.11 A	0.32 $\pm$ 0.10 A	0.40 $\pm$ 0.14 A
NO_3-N	0.38 $\pm$ 0.13 A	0.80 $\pm$ 0.12 B	0.35 $\pm$ 0.10 A
HA	68 $\pm$ 13.1 A	73 $\pm$ 13.1 A	62 $\pm$ 12.1 A
ETR	**80 $\pm$ 9.3 A**	**75 $\pm$ 9.8 A**	**51 $\pm$ 7.1 B**

Means followed by the same letter are not significantly different using Tukey-Kramer Honestly Significant Difference (HSD) post-hoc test ($\alpha = 0.05$).

Table 1.5. ETR selected physiochemical measurements for Clinch River tributaries, Virginia, and major land use categories.

Landuse Category	Tributary Name	TDS (g/L)	Al (mg/L)[a]	N03-N (mg/L)[a]	TP (mg/L)[a]
F	Indian Creek	0.067	0.0845	0.08	0.09
F	Middle Creek	0.150	0.0822	0.08	0.12
F*	Big Creek	0.150	0.0628	0.16	0.13
F	Big Stony Creek	0.050	0.0588	0.08	0.11
F	Cove Creek	0.117	0.0691	0.13	0.08
F	Stock Creek	0.217	<0.0525	0.08	0.03
A*	North Fork CR	0.117	0.0827	0.18	0.11
A*	South Fork CR	0.100	0.0891	0.23	0.09
A*	Cavitts Creek	0.217	0.0588	0.16	0.08
A	Little River	0.150	0.0541	0.21	0.13
A	Big Cedar Creek	0.167	0.0775	0.23	0.16
A*	Big Spring	0.233	<0.0525	0.23	0.10
A	Copper Creek	0.367	<0.0525	0.21	0.06
M*	Coal Creek	0.350	<0.0525	0.08	0.08
M	Swords Creek	0.167	0.0940	0.13	0.18
M	Dumps Creek	0.333	0.1715	0.10	0.12
M*	Lick Creek	0.300	<0.0525	0.08	0.11
M	Russell Creek	0.500	0.1253	0.05	0.10
M*	Guest River	0.283	0.0643	0.10	0.19
Forest		0.193	0.0683	0.10	0.09
Agriculture		0.125	0.0667	0.21	0.10
Mining		0.322	0.0934	0.09	0.13
All		0.213	0.0761	0.13	0.11
Developed		0.219	0.0644	0.15	0.11
Undeveloped		0.208	0.0838	0.13	0.11

[a]Concentrations in water column, n=1.
*Denotes developed (>1%) tributaries.

Table 1.6. Biological, toxicological and chemical parameter results with resulting ETRs for selected Clinch River tributaries, 2003.

Land use	Tributary	Biological SCI[a]	HA[b]	Toxicological CG[c]	DR[d]	ETR[e]
F	Indian Creek	81	72	1.16	71	86
F	Middle Creek	79	60	0.42	56	64
F*	Big Creek	90	73	0.74	53	78
F	Big Stony Creek	94	90	0.53	134	91
F	Cove Creek	84	57	0.57	72	79
F	Stock Creek	84	56	0.93	54	84
A*	North Fork CR	85	50	1.46	100	78
A*	South Fork CR	77	70	0.94	84	68
A*	Cavitts Creek	65	80	0.44	59	57
A	Little River	96	93	0.87	68	82
A	Big Cedar Creek	96	73	0.67	50	73
A*	Big Spring	82	77	1.01	47	79
A	Copper Creek	88	68	0.97	111	86
M*	Coal Creek	51	55	NR[f]	54	48
M	Swords Creek	69	64	0.31	108	51
M	Dumps Creek	54	50	1.06	90	44
M*	Lick Creek	51	60	1.37	79	56
M	Russell Creek	58	60	0.89	110	46
M*	Guest River	66	85	1.21	98	63
Forest		85	68	0.73	73	80
Agriculture		84	73	0.91	74	75
Mining		58	62	0.97	90	51
All		76	68	0.87	79	69
Developed		80	68	0.76	84	71
Undeveloped		71	69	1.02	72	66

[a]Stream Condition Index
[b]Habitat assessment (%)
[c]Clam growth (mm)
[d]*Daphnia* reproduction (x)
[e]Ecotoxicological Rating
[f]Not recovered
*Denotes developed (>1%) tributaries

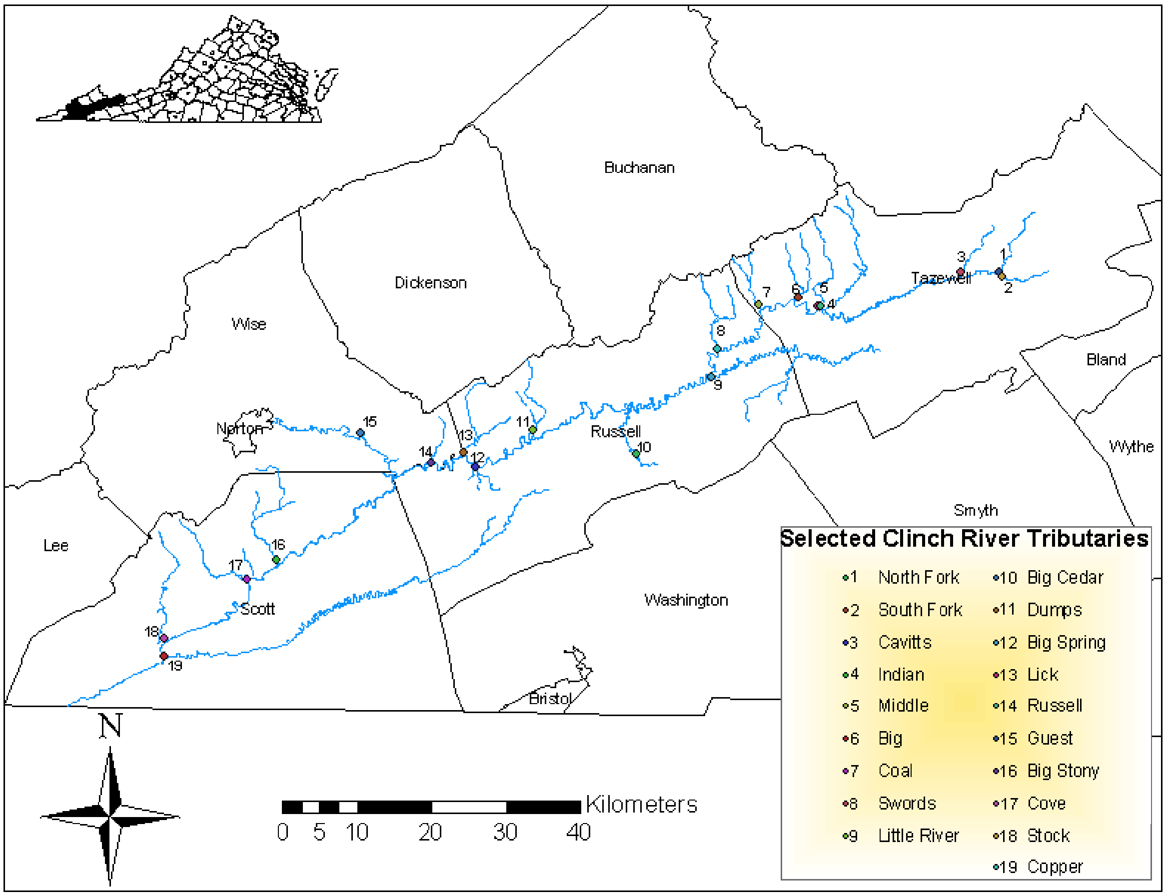

Figure 1.1. Upper Clinch River tributaries, Virginia, sampled and monitored for study.

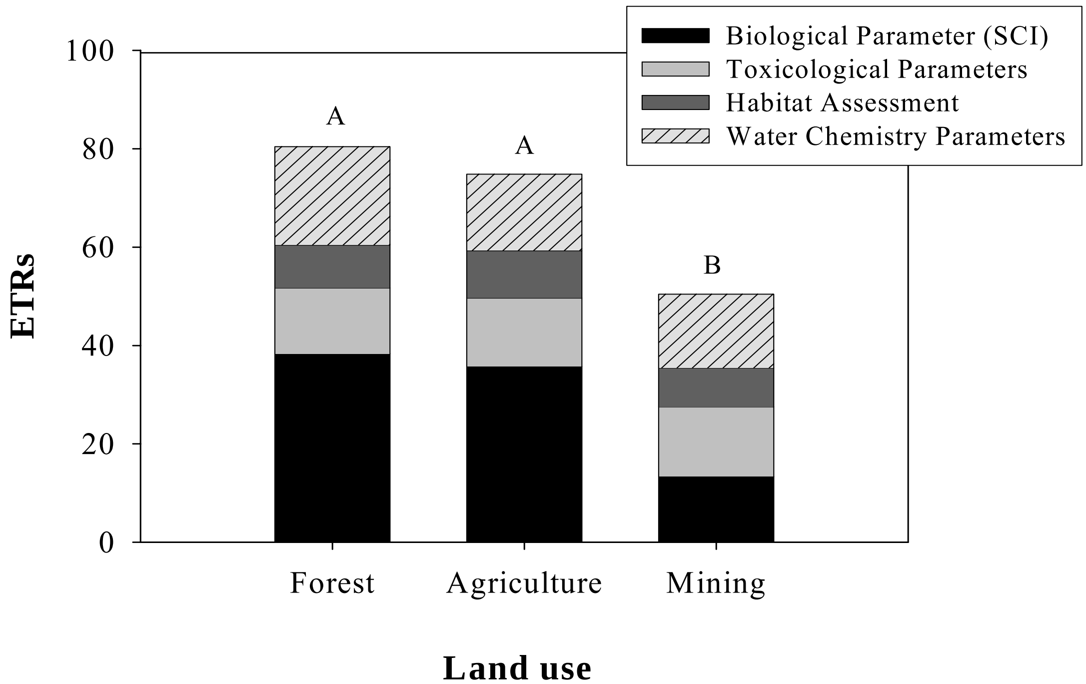

Figure 1.2a. Mean Ecotoxicological Ratings (ETRs) for Clinch River tributaries categorized by land use.

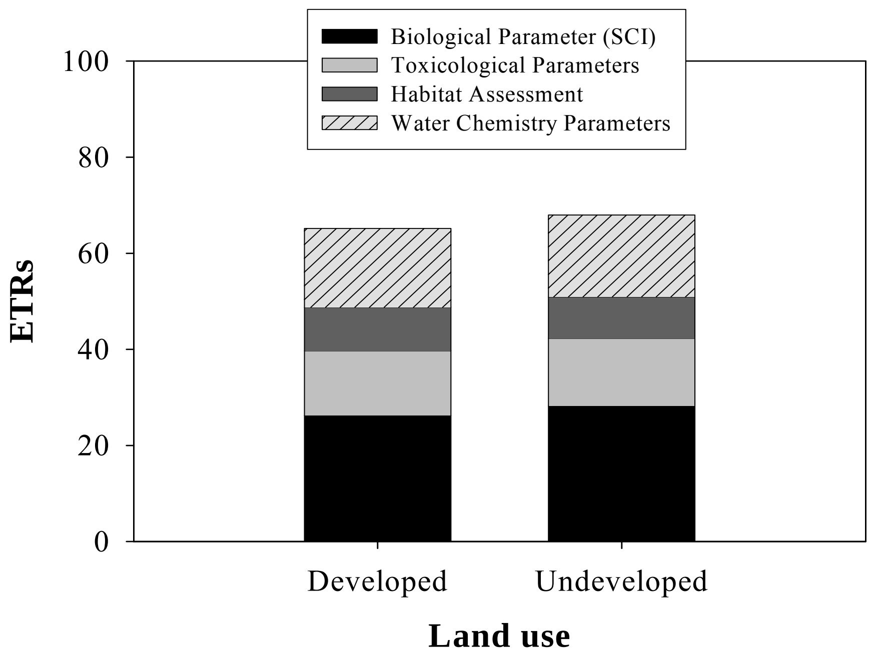

Figure 1.2b. Mean Ecotoxicological Ratings (ETRs) for Clinch River tributaries categorized by level of watershed developed.

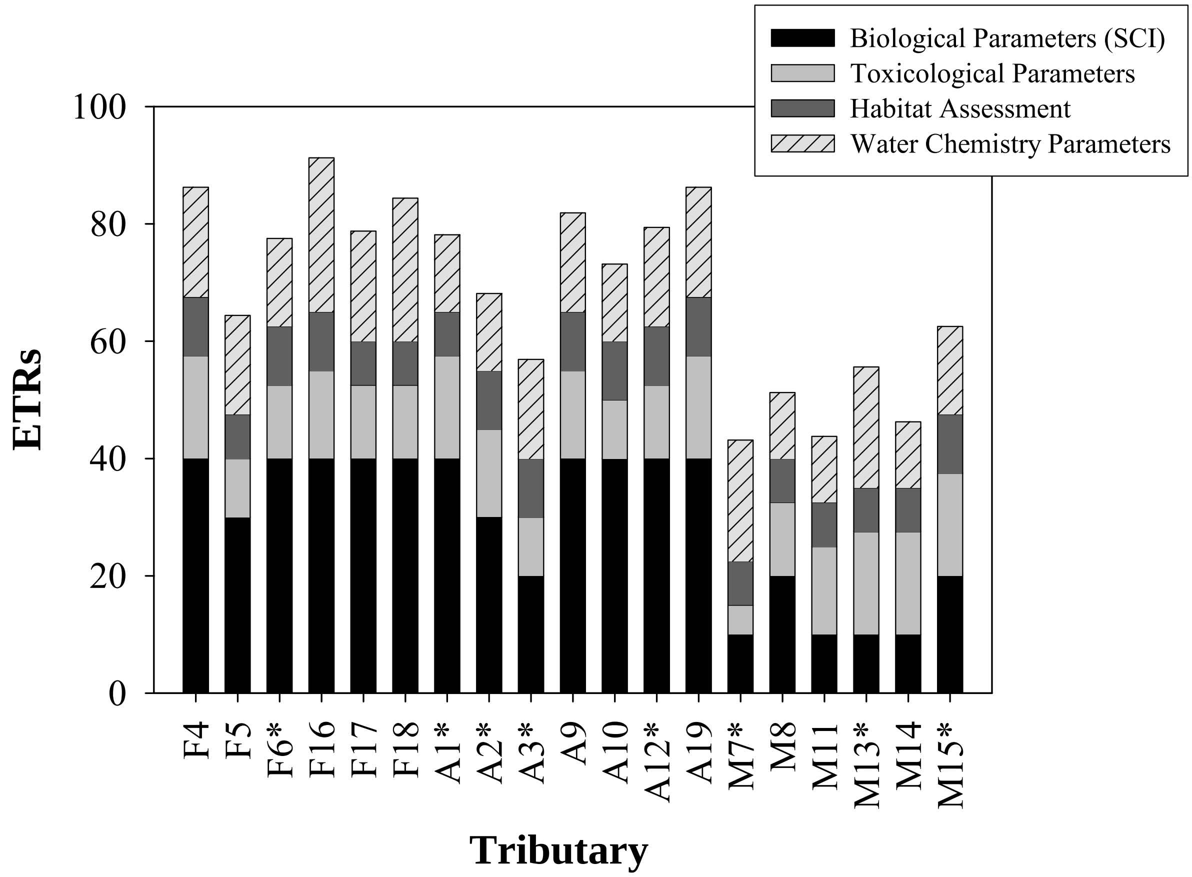

Figure 1.3. Ecotoxicological Ratings (ETRs) for Clinch River tributaries, Virginia.

Asterisks indicate developed tributaries (watersheds with >1% developed land).

CHAPTER 2. An Expanded Ecotoxicological Assessment of Nine Upper Clinch River Tributaries, Virginia.

Abstract: Although the existing assemblages are vanishing, the upper Clinch River in Virginia has one of the most diverse freshwater mussel communities in the world. Numerous studies have been conducted in the Clinch River mainstem to better understand unionid declines. While many speculate that pollution stressors, which largely originate from the tributaries of the upper Clinch River, may be adversely affecting mussel populations, few studies have been conducted in these major tributaries. This study is a continuation of previous work, which assessed aquatic health of select upper Clinch River tributaries based on biological, toxicological and chemical parameters. To gain further knowledge of the entire watershed, this ecotoxicological study was conducted on nine impaired tributaries of the upper Clinch River. For this 2004 study we used the same biological, toxicological and chemical parameters as before to better evaluate ecotoxicological health (via an Ecotoxicological Rating (ETR)). Using logistic regression, we did not find that predictor variables varied among land uses, years, or sites (land use, ETR $P = 0.8115$, SCI $P = 0.9812$; year, ETR $P = 0.9048$, SCI $P = 0.2815$; site, ETR $P = 0.9905$, SCI $P = 1.000$). Big Stony Creek had the highest ETR (93) of all sites sampled, analogous to the previous study in Chapter one. Downstream locations in Big Creek, Lick Creek, Big Cedar Creek, and North Fork Clinch River (CR) streams also had high ETRs (91, 82, 81, 81, respectively) while low scores were found in South Fork CR (60) and Big Spring (51). Upstream sites in Big Cedar Creek, Big Creek, Swords Creek, and the North Fork CR streams had high ETRs (86, 84, 84, 81, respectively) while Cavitts Creek, Big Spring and Lick Creek (61, 41, 41, respectively) had the lowest scores. Although not significant, variation among tributaries and between

years may be the result of high precipitation in one year and minimal stream degradation among streams sampled.

2.1. Introduction

Monitoring aquatic systems is necessary to understand natural and anthropogenic variation in system characteristics and assemblages. Numerous monitoring techniques have been developed (Richardson 1928; Chandler 1970; Hilsenhoff 1977; Sternberg et al. 1996; US EPA 2000; Cherry et al. 2001), but a universal monitoring technique remains elusive as each system is independent and varies with respect to precipitation, temperature, geology, land use and introduced stressors. It is particularly difficult to compare streams with multiple land uses because each land use introduces specific stressors to the system that affect biota in different ways. Agricultural land use contributes to stream bank erosion and runoff which increases sedimentation and nutrients, mostly nitrogen and phosphorous. Mining land use enhances runoff leading to increased total suspended/dissolved solids, and sedimentation. Further, mining land use can result in acidic conditions with elevated metals. To understand entire watersheds and ecoregions rather than individual streams, it is imperative that monitoring techniques incorporate differing land uses and any synergistic effects among specific land use stressors. Initial sampling indicated that tributaries of the upper Clinch River are likely suboptimal in regard to their potential influence on the mainstem Clinch River (Locke et al. In review). The objectives of this study were to further understand these catchments, from their headwaters to the confluence with the Clinch River; and to continue to monitor these tributaries to better understand specific land use factors that adversely influence their condition.

2.2. Materials and Methods

2.2.1. Study Site Selection and Sampling Sites

Several tributaries exhibiting suboptimal Ecotoxicological Ratings (ETRs) were not included in this study (defined in section 2.2.5 Ecotoxicological Rating). Tributaries selected for study were located in Tazewell, Russell and Scott Counties, Virginia (Figure 2.1). Tributaries with ETR <80 (generated in 2003) were selected for this study. Streams were chosen because previous study indicated these catchments were likely suboptimal, and require further assessment to accurately determine existing conditions (Table 2.1). Russell and Coal Creeks were not surveyed during this study because of inaccessibility and/or personal safety, and Middle and Dumps Creeks were dropped because in-depth studies have already been conducted. These assessments indicated that Dumps Creek is impaired at the headwaters due to active mining (Hull 2002); and Middle Creek is recovering from reclaimed abandoned mine land and acid-mine drainage (AMD) seeps (Merricks 2004). Furthermore, both creeks have current Total Maximum Daily Loads (TMDLs) (US EPA 2003). Russell Creek was not included in this study because of inaccessibility due to active mining permits, three in the mainstem (permit numbers (PN) 1201475, 1803869, and 1803868) and two in the tributaries of the creek (PN 1101580 and 1201862) (Former VA DMLR employee Claire Trent, personal communication, August 2003). Inaccessibility and personal safety hindered further studies in Coal Creek where active permits exist in the upper watershed. Russell and Coal creeks were assumed to be impaired from their headwaters to their confluence with the Clinch River based on their condition at the confluence and the fact that active mining is present in the upper watershed. This is further supported by several studies which suggest mining has a negative influence on stream health (Clements 1994; Soucek et al. 2000; Cherry et al. 2001; Schmidt et al. 2002; Locke et al. In review).

Downstream sampling sites utilized in previous surveys were maintained and sampling was conducted within 1.5 km upstream of each tributary confluence with the Clinch River (except for Big Cedar Creek and Guest River, sampled approximately 5.5 km and 8.0 km upstream from the confluence, respectively, because of accessibility). Upstream sites were chosen based on accessibility and sampled at the furthermost point upstream of the confluence where there is continuous flow throughout the year and on comparable habitat. Each sampling reach incorporated approximately 100-200 m, depending on stream size and site accessibility.

2.2.2. Water Chemistry Analysis

Water samples were collected as grab samples from the stream center, preserved on ice (4°C), transported to Virginia Polytechnic Institute and State University (VPI), and acidified (APHA 1995). Collection, transportation and analysis did not exceed 48 hours. Trace metal analysis was conducted at the VPI Soil Testing Laboratory using inductively coupled plasma (ICP) spectrometry (APHA 1995). Ions analyzed included Al, Cu, Mn, Fe, and Zn. Total phosphorous (TP) and nitrates were analyzed at the VPI Biological Systems Engineering Water Quality Testing Laboratory using the APHA 1995 protocol. Total dissolved solids (TDS) were analyzed according to APHA Standard Methods (1995).

2.2.3. Benthic Macroinvertebrate Sampling and Habitat Assessment

Qualitative sampling was conducted on May 24-25, 2004 according to Barbour et al. (1999) using the 20-kick method in conjunction with habitat assessment. Due to flooding on May 25th, Big and Swords Creek could not be sampled and were later sampled on July 7th.

Four replicate qualitative samples were obtained utilizing 800-µm mesh D-framed nets (Wildco 425-D10) and preserved in 95% ethanol until processed and identified to the lowest practical taxonomic level (usually genus) using standard keys (Merritt and Cummins 1996). The West Virginia Stream Condition Index (SCI) was utilized as the macroinvertebrate parameter in the ETR to maintain consistency among studies (US EPA 2000; Locke et al. In review), and habitat assessment was conducted in conjunction with benthic sampling according to the US EPA Rapid Bioassessment Protocols (RBPs) (Barbour et al. 1999).

2.2.4. Toxicological Testing

Sediment tests were conducted according to procedures in the American Society of Testing and Materials (ASTM 1995) and Nebeker et al. (1984) with modifications including an overlying reference water (Sinking Creek, Giles Co. VA) used by the Ecotoxicology Laboratory at VPI. Toxicity tests were conducted in 250-mL clean, glass beakers with approximately 75 mL sediment volume and 150 mL of reference water. Four replicates with four *Daphnia magna* in each replicate were subjected to tributary sediments for 10 days. Overlying water was changed daily and dissolved oxygen (DO_2) was monitored daily to ensure adequate DO_2. Following water renewal *Daphnia* were fed a daily diet of the green algae, *Selenastrum capricornutum*. Endpoint measurements were survivorship and reproduction.

Asian clams (*Corbicula fluminea)* for *in situ* toxicity tests were collected from the New River approximately one mile upstream from Big Falls at McCoy, Virginia. Organisms were maintained in Living Streams® (Frigid Units, Toledo, OH), and fed a daily diet of *Neochloris sp.* before being placed into the tributaries. Clams were measured from their umbo to their ventral margin with ProMax Fowler NSK ® digital calipers (Fowler Co. Inc., Boston, MA, USA). Only clams between 8.0 mm and 12.0 mm were selected for actual test organisms. Test organisms were placed in 18 cm by 36 cm polypropylene mesh bags with a mesh size of ~0.5 cm^2. Five separately marked bags with five organisms in each bag were placed in each upstream and downstream site in the ten tributaries (except for Big Stony where only a downstream site was assessed) for 50 days. Bags were secured in pools located downstream from riffle areas to ensure adequate DO_2 supply. When no pool existed, best professional judgment was used to choose comparable habitat. Mesh bags were identified as the experimental unit and a mean size was determined for each bag. Each site was represented by five (i.e., n=5/tributary) growth measurements, each derived from changes in mean clam length within each bag. Clam growth served as the endpoint measurement. The North Fork Clinch River was used as the reference site for comparisons and to maintain consistency among studies.

2.2.5. Ecotoxicological Rating

An ETR was devised utilizing eight parameters with the purpose of ranking the tributaries from least to most favorable for aquatic life (Table 2.2). This system included a benthic macroinvertebrate metric (SCI), two toxicological indicators *(in-situ* growth of Asian clams and *D. magna* reproduction), four water chemistry parameters (total dissolved solids, aluminum, nitrate, and total phosphorous) and habitat assessment. Final ETRs were based on a 100-point system following Soucek et al. (2000), Cherry et al. (2001), and Schmidt et al. (2002). Except for Al, all parameter values (SCI, *D. magna* reproduction, clam growth, habitat assessment, TDS, TP and NO_3^--N) were calculated by dividing the tributary value by the reference value (North Fork for clam growth, Stock Creek for TP and Big Stony Creek for other parameters). Aluminum values, however, were segregated into representative ranges to present degrees of potential impairment based on US EPA Water Quality Criteria (WQC) which were not divided by the reference value. The transformed parameter values, now a percentage of the reference (except for Al), were placed into one of five categorical ranges so that each parameter for each tributary received 0, 2.5, 5, 7.5 or 10 points. These scores were then multiplied by the ETR weight for a final parameter score. ETR weights were designed as follows: biological and toxicological indicators represented 60% of the system, (macroinvertebrates 40%, *Daphnia* reproduction in sediment tests 10%, *in situ Corbicula* growth 10%), water chemistry represented 30% (Al, TP, NO_3^--N and TDS each at 7.5%), and habitat assessment accounted for the remaining 10%. All eight parameter scores were summed for the final ETR. This system was identical to the previous year and was used to address comparisons between upstream and downstream sites for each individual tributary, as well as between years.

The WVA SCI, which incorporates six macroinvertebrate metrics into one numerical value, was utilized in the ETR to represent the benthic macroinvertebrate communities (US EPA 2000). This index weighs the importance of different groups using counts and pollution tolerance, and was weighted more heavily than other parameters in the ETR. Benthic macroinvertebrates demonstrate greater environmental realism and are indicative of stream health in this system. Further, water chemistry parameters were sampled on one occasion and thus do not represent the temporal variability that is inherent in water quality observations. For these reasons ETR parameters were not weighed equally to determine stream quality, but were identical to parameter weights devised in 2003.

Chronic *D. magna* sediment reproduction and *C. fluminea in situ* growth were used as toxicological indicators. While five metals were analyzed, only aluminum concentrations were used in the ETR because Cu, Fe, Mn, and Zn were either below wavelength detection limits when analyzed, and/or below any acute and/or chronic toxicity thresholds. Total phosphorous and NO_3^--N were included to represent potential agricultural influences within the watershed. Although US EPA (2002) reference values for the appropriate ecoregion XI: 67 (25^{th} percentile of all data: TP = 0.010 mg/L, and NO^-_3-N= 0.093 mg/L) were considered for differentiating between streams, they were not used in the ETR parameter scaling so that the ETR methodology was identical for both sampling years. Total dissolved solids, which have been shown to cause impairment in benthic assemblages and laboratory bioassays at elevated concentrations (Kennedy et al. 2003), were used in the ETR. Sources of total dissolved solids include treated wastewater effluents and mining (Goodfellow et al. 2000; Kennedy et al. 2003).

2.2.6. Land Use

Land use categories were maintained from the previous study with slight modifications (Locke et al., In review, Table 2.3). These categories were derived from Geographic Information Systems (GIS) software (ArcView 8.3) and National Land Characterization Data (USGS 2003) which were used to analyze and quantify land use within the watershed of each tributary. The percentage of each land use category within each tributary was determined. Based on these percentages, we categorized each tributary as belonging to one of two major categories, agricultural (A) or mining (M) influenced (Locke et al, in review). Although these methods categorized Big Creek as forest influenced, upstream Big Creek had the second highest Al concentration (over three times higher than acceptable water quality criteria, WQC), indicating mining influence. Big Creek is a recently recovered acid-mine drainage (AMD) watershed based upon the newly landscaped recovery.

Between 2003 and 2004 AMD remediation was only initialized and most likely attributed to the varying aluminum concentrations. Big Creek was therefore categorized as a mining influenced stream.

2.2.7. Statistical Analysis

Nine streams were selected for analysis [agricultural (n=5), mining (n=4)]. Big Stony was used as the reference site, but not included in statistical analyses. We used logistic regression models to test whether predictor variables (ETR, SCI, toxicological parameters: *Daphnia* reproduction and clam growth, and chemical parameters: TP, NO_3^--N, Al, TDS) varied between land uses, years, or up and downstream sites because sample size was small and data was not normal (Cox and Snell 1970; Hosmer and Lemeshow 1989). Land use regression models tested the probability that ETR and SCI significantly varied between agriculture or mining tributaries, and the year regression models tested whether ETR and SCI varied between 2003 or 2004 (Table 2.4). The site regression models examined variation in ETR, SCI, the toxicological parameters (DR and CG), and the chemical parameters (TP, NO_3^--N, Al, TDS) in relation to upstream or downstream sites.

2.3. Results

2.3.1. Site

2.3.1.1. Water Chemistry

The logistic regression model indicated that chemical parameters did not vary between up and downstream stream sites (Table 2.4). Regarding TDS, the Guest River (0.533 g/L) and Lick Creek (0.317 g/L) had the highest upstream values while Lick (0.267 g/L) and Cavitts Creeks (0.25 g/L) had the highest ones downstream (Table 2.5). Low TDS was found in South Fork CR, Big Cedar Creek and Cavitts Creek (0.117, 0.183 and 0.1833 g/L, respectively) in upstream sites, and in Big Stony Creek and Big Cedar Creek (0.05 and 0.117 g/L, respectively) in downstream sites. The highest aluminum concentrations, above US EPA WQC (0.087 mg/L) (US EPA 1988), were found in the upstream site of Big Creek (0.2999 mg/L) and both upstream and downstream sites of Swords Creek (0.2217 and 0.692 mg/L, respectively). High aluminum concentrations, also above US EPA WQC, were found in Big Cedar Creek and North Fork CR (0.1534 and 0.0921 mg/L, respectively).

High NO_3^--N values for upstream sites were measured in Big Spring, Big Cedar Creek, and South Fork CR (0.34, 0.26, and 0.26 mg/L respectively), and the downstream sites of Big Cedar Creek, Big Spring, Cavitts Creek, and South Fork CR (0.23, 0.26, 0.26, and 0.34 mg/L respectively) (Table 2.5). High NO_3^-N concentrations in upstream sites were in Lick Creek, North Fork CR, and South Fork CR (0.24, 0.22, and 0.2 mg/L, respectively) and low values were in Cavitts Creek (0.1 mg/L) and Big Cedar Creek (0.11 mg/L). Downstream sites of South Fork CR, North Fork CR and the Guest River had high inputs of 0.27, 0.17, 0.17 mg/L, respectively, while low concentrations were measured in Big Spring (0.11 mg/L) and Lick Creek (0.12 mg/L).

2.3.1.2. Biological Parameter, Habitat Analysis and Toxicological Parameters

The logistic regression model did not indicate that up and downstream stream sites differed in SCI scores (Table 2.4), and mean SCI's were comparable among agricultural and mining influenced tributaries for both upstream and downstream sites for 2004 (Table 2.6). The reference site (Big Stony Creek) had a slightly lower SCI in 2004, although it's score (83) and Big Cedar Creek (88) had the highest SCI's for downstream sites. Big Spring had the lowest SCI for both downstream (40) and upstream (39) sites, while Lick Creek and

Cavitts Creek also received low marks in the upstream sites (40 and 46, respectively), and South Fork CR (57) in the downstream sites.

Regarding *in situ* Asian clam tests, growth was greatest in upstream sites of Big Creek (1.19 mm) and Big Cedar Creek (0.7 mm) and lowest in the North and South Fork CR (0.11 and 0.15 mm, respectively) (Table 2.6). Clams in downstream sites of Big Spring and the North Fork CR grew the most (0.85 and 0.84 mm) while those in Cavitts Creek (0.11 mm), Big Stony Creek (0.27 mm) and the South Fork CR (0.27 mm) grew the least. Upstream sites of Lick Creek and Big Spring and downstream sites of Cavitts Creek and Lick Creek had high mean *Daphnia* reproduction (112, 93, 120, and 103 neonates, respectively), while low mean reproduction responses to sediments were found in South Fork CR (41) and Big Creek (53) for upstream sites and in Swords Creek (67), South Fork CR (73) and the Guest River (74). Logistic regression model did not indicate that the toxicological parameters differed between up and downstream stream sites (Table 2.4).

2.3.1.3. Ecotoxicological Rating

The logistic regression model did not indicate that the ETR varied between up or downstream stream sites (Table 2.4 and Figure 2.2). Big Stony Creek had the highest ETR (93), while mining influenced streams, Big Creek and Lick Creek, and agricultural influenced streams, Big Cedar Creek and North Fork CR, also had high scores for downstream sites (91, 82, 81, and 81, respectively) (Table 2.6). Big Cedar Creek, Big Creek, Swords Creek and North Fork CR had upstream high scores of 86, 84, 84, and 81, respectively, and although not significant, ETRs in downstream sites were lower (Big Spring, 51 and South Fork CR, 60) and low in upstream sites of Lick Creek (41) and Big Spring (41).

2.3.2. Year

2.3.2.1.Water Chemistry

Mean TDS values between sampling years (2003 and 2004) varied the most in agricultural streams (0.167 and 0.200 mg/L, respectively) (Table 2.7). Aluminum values for mining influenced catchments varied between 2003 (0.0684 mg/L) and 2004 (0.2192 mg/L). Nitrate-N values remained virtually constant for all land uses among sampling years, while mean total phosphorous concentrations were moderately constant for land uses between sampling years.

2.3.2.2.Biological Parameter, Habitat Analysis and Toxicological Parameters

SCIs did not vary between sampling years (Table 2.4). Although mean SCI's for mining influenced tributaries remained the same, SCIs in agricultural influenced streams did vary from 81 in 2003 to 63 in 2004 (Table 2.8). Mean habitat scores among land uses were consistent between years. Growth in *Corbicula* was greater in 2003 compared to 2004 among agricultural and mining influenced tributaries. Mean *Daphnia* reproduction in agricultural tributary sediment tests varied the most between years (2003: 69, 2004: 93).

2.3.2.3.Ecotoxicological Rating

Overall, ETRs did not vary between sampling years (Table 2.4 and Figure 2.3). However, there was variation among the mining influenced tributaries (2003: 57 and 2004: 79), but not among agricultural catchments (2003: 71 and 2004: 70) (Table 2.8). In 2003, South Fork CR and Big Spring had higher ETRs compared to 2004. In 2004, ETRs were greater for North Fork CR, Cavitts Creek, Big Cedar Creek, Big Creek, Swords Creek, Lick Creek, the Guest River and Big Stony Creek. The highest ETR in 2003 was in Big Stony Creek (91) and in 2004, high ETRs were found in Big Stony Creek (93), Big Creek (91), Big

Cedar Creek (86), Lick Creek (82) and North Fork CR (81). The lowest ETRs in 2003 were Swords Creek (51) and Cavitts Creek (57), while 2004 lowest scores were Big Spring (51) and South Fork CR (60).

2.3.3. Land use

2.3.3.1. Water Chemistry

Mean TDS and aluminum values were highest in mining influenced tributaries (0.269 mg/L vs. 0.1822 mg/L) compared to agricultural streams (0.195 mg/L vs. 0.0633 mg/L) (Table 2.5). Mean nitrate-N levels were highest in agricultural streams (0.23 mg/L). Mean total phosphorous concentrations were slightly higher in agricultural streams.

2.3.3.2. Biological Parameter, Habitat Analysis and Toxicological Parameters

The SCI did not vary between land uses (Table 2.4), and mean habitat scores among land uses were lowest in agricultural streams in 2004 (Table 2.6). Mean *Corbicula in situ* growth was more enhanced in mining influenced tributaries (0.64 mm) compared to agricultural influenced streams (0.43 mm in Table 2.8). Mean *Daphnia* reproduction in sediment tests was greatest in agricultural streams for 2004.

2.3.3.3. Ecotoxicological Rating

The ETRs did not vary between land uses (Table 2.4 and Figure 2.4). Relative to up and downstream sites within land use categories, mean ETRs for agricultural streams were identical (69 in Table 2.6). Mean ETRs in mining tributaries' were higher in the downstream sites (82) compared to upstream (71). Although not significant, mean up and downstream site ETRs for mining catchments were higher (76) compared to agricultural streams (69). In 2003, mean ETRs for mining influenced tributaries were lower than in agricultural influenced streams (57 and 71, respectively) (Table 2.8). However, for 2004, mean ETRs were highest in mining influenced tributaries (76) compared to those influenced by agriculture (69). Collectively, 2003 and 2004 mean ETRs for mining streams (69) were lower than agricultural catchments (71). Incorporating both year and sites, land use mean ETRs varied the most in mining influenced tributaries (Tables 2.6 and 2.8).

2.4. Discussion

All parameters examined did not vary between land uses, sites and years, probably due to the small sample sizes and substantial variation in one agricultural stream (Big Spring) and one mining stream (Lick Creek). Further, results from the analysis of the least ecotoxicologically healthy streams is not surprising given that the lowest scoring mining tributaries and the highest scoring agricultural streams were removed. Thus, it is reasonable that the remaining tributaries used in analyses were comparable. Differences within streams between sites for a few tributaries would have been significant if replication had been conducted for each parameter. Statistical analysis for differences between upstream and downstream sites could not be performed for each individual tributary because the eight responses were measured only once.

Variation in ETRs among years was attributed to multiple factors. Foremost, rain events were more frequent during 2004 and may be responsible for variation in SCI scores, which was the most heavily weighted component of the ETR. Precipitation averages in May were above normal with only three days of no rain. Tazewell, Russell and Lee counties were issued a disaster declaration as a result of flooding that occurred in late May which resulted in mudslides and sewage in flood water (Hawkins, Bluefield Daily Telegraph, June 16, 2004). While sampling occurred just before flooding for all but two tributaries (Big and Swords Creek later sampled on July 7th), the macroinvertebrate communities may have not been thoroughly represented in sampling because many were not there. Benthic macroinvertebrates are known to retreat to the hyporheic zone during times of high flow/floods (Resh et al. 1988; Allan 1995) and because of drift (Brittain and Eikeland 1988; Impert and Perry 2000). Big and Swords Creeks, sampled after flood events, had substantial improvements in bug communities in 2004 compared to that in 2003, revealing more robust communities within these streams which may have been augmented by rain events, due to drift. It also should be noted, when considering the impact of excessive precipitation, that tributaries are not consistent in size, order, and discharge, which may have contributed to the variation in benthic macroinvertebrates within their respective drainages.

Other spurious factors (sampling format, changes in stream flow pattern) may have attributed to the variation seen in macroinvertebrate communities. Although methodology was consistent, three individuals carried out sampling in 2003 whereas a single surveyor did so in 2004. Also attributing to the variation observed between years was the fact that pools rarely existed in upstream sites and the majority of sampling took place in riffles and runs so that habitat in all sites would be comparable. This may explain variation in SCI metrics for Big Spring (2003 compared to 2004), as well as the fact that Big Spring is a spring and has a different macroinvertebrate assemblage than other streams included in this study. In our previous study, some sampling reaches incorporated undesirable habitat, so for this study we sampled adjacent reaches with preferable habitat for biota. Also noteworthy is the fact that external influences may have been occurring with volatile frequencies, durations, components (nutrients and/or heavy metals) and concentrations (of constituents) that may not be represented in a single sample. This is especially critical in agricultural streams, contributing non-point source pollution, where variation in individual parameters was greatest between years and among land uses. Further, drainage area and stream discharge most likely had a role in the variability between sites. This is supported by stream theory (Vannote et al. 1980) and studies by Norton et al. (2000) who found that biological and abiotic variables were significantly correlated with drainage area. Also contributing to the variability was the reference site, Big Stony Creek, which had a lower SCI for macroinvertebrates for 2004 compared to 2003. Because the SCIs were calculated using the percent of the reference, the biological parameter for some tributaries was placed into a higher range bracket in 2004.

Daphnia reproduction in response to tributary sediments as well as TDS and TP varied the most in agricultural influenced streams between years among land uses. This aspect indicates that agricultural streams may be negatively affected in varying episodes and durations compared to mining influenced tributaries where variability among response parameters was nominal except for aluminum concentrations (Table 2.7) and the overall ETR (Table 2.8). The mean ETRs for mining influenced tributaries was inflated in 2004 because Lick and upstream Swords Creeks had high macroinvertebrate scores. *Corbicula* growth varied in both agricultural and mining influenced streams between years, which could have been influenced by different exposure times. Clams in 2003 were *in situ* for 10 days longer than in 2004. Lower temperatures during exposure times in 2004 may also have accounted for variation seen in growth between sampling years. Further, aluminum values varied considerably between years in mining influenced tributaries (Table 2.7) suggesting that trace metal frequencies, durations and concentrations may also be varying within the contributors of mining influenced tributaries. Excessive runoff from frequent rain events also could have been responsible for increased aluminum values seen most notably in Swords and Big Creeks.

Variation was greatest among upstream and downstream sites within the mining influenced tributaries, such as Lick Creek in 2004 (Table 2.5 and Table 2.6). Upstream sites of Lick Creek, Big Creek and the Guest River had lower ETRs than their respective downstream sites, which is indicative of mining influences in the upper part of these watersheds. Swords Creek had a lower ETR in the downstream site suggesting that the mining influences originate between the upper vs. lower sites and are negatively affecting the subwatershed. Except for South Fork CR and Cavitts Creek, upstream and downstream sites in agricultural streams were comparable. The downstream site in the South Fork CR had a lower ETR relative to the upstream site because of a lower SCI score, suggesting the macroinvertebrate community may have been negatively affected by land use within this stream. The lower ETR for the upstream site of Cavitts Creek was also attributed to a lower SCI score, although most likely due to habitat. The headwater of Cavitts Creek is a lake, and the accessible sampling area just below the lake is suboptimal, compared to the downstream site. The upstream site is not indicative of conditions in Cavitts Creek, and low ETRs among years is most likely a result of the contributing influences the stream receives as it flows into the Clinch River.

2.5 Conclusions

The results of this study indicate that further research needs to be conducted in the Clinch River tributaries to assess the aquatic health of entire respective catchments. Also, studies incorporating sampling reaches in the mainstem need to be addressed to understand the effects that tributaries have on the mainstem. The majority of the streams had higher

ETRs in 2004 compared to 2003 suggesting that variation in biotic responses as well as measured constituents may play a role in shifting environmental conditions. This may be influencing freshwater mussel declines since they are sensitive to irregular environmental changes. However, no conclusions can be made regarding the impact these tributaries have on aquatic biota in the mainstem because no sampling reaches were conducted within the Clinch River.

2.6. Acknowledgements

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Table 2.1. Selected Clinch River tributaries, Virginia, and explanation for the study in 2004.

	Tributary	Reason(s) for expanded assessment of entire catchments
1	North Fork	2003 ETR 78, headwaters of Clinch River, flows through town of Tazewell.
2	South Fork	2003 ETR 68, headwaters of Clinch River.
3	Cavitts Creek	2003 ETR 57, petroleum and recycling center along stream bank.
4	Big Creek	2003 ETR 78, runoff from houses, waste water treatment plant, and reclaimed mine lands.
5	Swords Creek	2003 ETR 51, active and abandoned mining.
6	Big Cedar	2003 ETR 73, waste water treatment plant.
7	Big Spring	2003 ETR 79, straight-pipe septic systems.
8	Lick Creek	2003 ETR 56, active and abandoned mining.
9	Guest River	2003 ETR 63, active mining, abandoned mining, AMD and coal storage piles. Flows through coal housing facilities in Norton, VA.
10	Big Stony Creek	Reference stream. 2003 ETR 91.

Table 2.2. Developed ranges with corresponding points used in the ETR.

	Allotted Points in ETR					
	0	2.5	5	7.5	10	
Parameter			Range			% of System
SCI[a]	0-49	50-59	60-69	70-79	80-100	40
DR[b]	0	1-25	26-50	51-75	76-100	10
CG[c]	0	1-25	26-50	51-75	76-100	10
HA[d]	0	1-25	26-50	51-75	76-100	10
TDS[e]	0	1-25	26-50	51-75	76-100	7.5
Al[f]	0.0900-.0999	0.0800-.0899	0.0700-.0799	0.0600-.0699	0.0500-.0599	7.5
TP[gh]	0	1-25	26-50	51-75	76-100	7.5
Nitrate-N[h]	0	1-25	26-50	51-75	76-100	7.5
Total						100

[a]Stream Conditon Index

[b]*Daphnia* reproduction

[c]Clam growth (mm)

[d]Habitat assessment

[e]Total dissolved solids (g/L)

[f]Aluminum (mg/L)

[g]Total phosphorous

[h]mg/L

Table 2.3. Distribution of major land uses within tributary watersheds[a] and each tributary's major land-use influence classification.

Tributary	Percent Forest[a]	of Agriculture[a]	Watershed Developed[a]	Area Mining[a]	Category[b]
1. North Fork	54.5	43.2	2.0	0.1	A
2. South Fork	54.1	44.1	1.6	0.2	A
3. Cavitts Creek	64.4	33.9	1.1	0.0	A
6. Big Cedar Creek	71.1	27.9	0.9	0.0	A
7. Big Spring	64.8	32.6	2.5	0.1	A
4. Big Creek	65.6	1.5	1.2	1.6	M
5. Swords Creek	91.4	5.4	0.3	2.7	M
8. Lick Creek	92.5	4.7	1.2	1.5	M
9. Guest River	78.9	5.6	5.7	9.0	M
10. Big Stony	98.4	1.4	0.0	0.1	F

[a]Percentages do not add up to 100 because lands in other land use categories are not represented.

[b]F = Forest, A = Agriculture, M = Mining.

Table 2.4. Model likelihood ratios for logistic regression models used to test whether variables predicted land use, year or site. Variables used were the Ecotoxicological Rating (ETR), Stream Condition Index (SCI), clam growth (CG) (mm), *daphnia* reproduction (DR), total dissolved solids (TDS) (g/L), aluminum (Al) (mg/L), nitrate-N (NO_3^-N) (mg/L), and total phosphorous (TP) (mg/L).

	Model likelihood ratio		
Land use models	**Chi-Square**	**DF**	**P-Value**
ETR	0.4177	2	0.8115
SCI	0.0379	2	0.9812
Year models			
ETR	4.0981	9	0.9048
SCI	10.916	9	0.2815
Site models			
ETR	2.0604	9	0.9905
SCI	0.3175	9	1.0000
CG and DR	17.0163	10	0.0740
TDS, Al, NO_3-N, TP	8.4195	12	0.7515

Table 2.5. Selected ETR physiochemical measurements for Clinch River tributaries, Virginia, upstream and downstream sites (2004), and major land use categories.

Landuse Category	Tributary Name	Sampling Site	TDS (g/L)	Al (mg/L)[a]	N03-N (mg/L)[a]	TP (mg/L)[a]
A	North Fork CR	upstream	0.200	0.0389	0.13	0.22
		downstream	0.217	0.0921	0.18	0.17
A	South Fork CR	upstream	0.117	0.0634	0.26	0.20
		downstream	0.200	0.0609	0.23	0.27
A	Cavitts Creek	upstream	0.183	0.0281	0.10	0.10
		downstream	0.250	0.0549	0.26	0.16
A	Big Cedar Creek	upstream	0.183	0.0715	0.26	0.11
		downstream	0.117	0.1534	0.34	0.15
A	Big Spring	upstream	0.267	0.0313	0.34	0.12
		downstream	0.217	0.0388	0.26	0.11
M	Big Creek	upstream	0.300	0.2999	0.05	0.13
		downstream	0.183	0.0790	0.16	0.13
M	Swords Creek	upstream	0.200	0.2217	0.10	0.12
		downstream	0.133	0.6920	0.10	0.15
M	Lick Creek	upstream	0.317	0.0334	0.13	0.24
		downstream	0.267	0.0395	0.08	0.12
M	Guest River	upstream	0.533	0.0259	0.16	0.14
		downstream	0.217	0.0663	0.16	0.17
Agriculture		upstream	0.190	0.0466	0.22	0.15
		downstream	0.200	0.0800	0.25	0.17
Mining		upstream	0.338	0.1452	0.11	0.16
		downstream	0.200	0.2192	0.13	0.14
Agriculture		All	0.195	0.0633	0.24	0.16
Mining		All	0.269	0.1822	0.12	0.15

[a]Concentrations in water column, n=1.

Table 2.6. Biological, toxicological and chemical parameter results with resulting ETRs for selected Clinch River tributaries for upstream and downstream sites, 2004.

			Biological		Toxicological		
Land use	Tributary	Site	SCI[a]	HA[b]	CG[c]	DR[d]	ETR[e]
A	North Fork CR	upstream	94	73	0.11	69	81
		downstream	71	63	0.84	92	81
A	South Fork CR	upstream	79	66	0.15	41	76
		downstream	57	68	0.27	73	60
A	Cavitts Creek	upstream	46	56	0.45	91	61
		downstream	66	62	0.10	120	73
A	Big Cedar Creek	upstream	83	61	0.70	89	86
		downstream	88	63	0.60	87	81
A	Big Spring	upstream	39	55	0.23	93	41
		downstream	40	61	0.85	90	51
M	Big Creek	upstream	81	84	1.19	53	84
		downstream	76	77	0.76	95	91
M	Swords Creek	upstream	79	75	0.58	57	84
		downstream	60	71	0.72	76	76
M	Lick Creek	upstream	40	54	0.32	112	41
		downstream	61	72	0.62	103	82
M	Guest River	upstream	59	61	0.27	67	73
		downstream	62	78	0.68	74	79
Agriculture		upstream	68	62	0.33	77	69
		downstream	64	63	0.53	92	69
Mining		upstream	65	69	0.59	72	71
		downstream	65	75	0.70	87	82
Agriculture		All	66	63	0.43	85	69
Mining		All	65	72	0.64	80	76

[a]Stream Condition Index
[b]Habitat assessment (%)
[c]Clam growth (mm)
[d]*Daphnia* reproduction (x)
[e]Ecotoxicological Rating

Table 2.7. Selected ETR physiochemical measurements for Clinch River tributaries, Virginia, sampling seasons 2003 and 2004, and major land use categories.

Landuse Category	Tributary Name	Sampling Year	TDS (g/L)	Al (mg/L)[a]	N03-N (mg/L)[a]	TP (mg/L)[a]
A	North Fork CR	2003	0.117	0.0827	0.18	0.11
		2004	0.217	0.0921	0.18	0.17
A	South Fork CR	2003	0.100	0.0891	0.23	0.09
		2004	0.200	0.0609	0.23	0.27
A	Cavitts Creek	2003	0.217	0.0588	0.16	0.08
		2004	0.250	0.0549	0.26	0.16
A	Big Cedar Creek	2003	0.167	0.0775	0.23	0.16
		2004	0.117	0.1534	0.34	0.15
A	Big Spring	2003	0.233	<0.0525	0.23	0.10
		2004	0.217	0.0388	0.26	0.11
F	Big Creek	2003	0.150	0.0628	0.16	0.13
M		2004	0.183	0.0790	0.16	0.13
M	Swords Creek	2003	0.167	0.0940	0.13	0.18
		2004	0.133	0.6920	0.10	0.15
M	Lick Creek	2003	0.300	<0.0525	0.08	0.11
		2004	0.267	0.0395	0.08	0.12
M	Guest River	2003	0.283	0.0643	0.10	0.19
		2004	0.217	0.0663	0.16	0.17
F	Big Stony Creek	2003	0.050	0.0588	0.08	0.11
		2004	0.050	0.0235	0.08	0.16
Agriculture		2003	0.167	0.0721	0.21	0.11
		2004	0.200	0.0800	0.25	0.17
Mining[b]		2003	0.250	0.0703	0.10	0.16
		2004	0.200	0.2192	0.13	0.14
Agriculture		All	0.184	0.0761	0.23	0.14
Mining[b]		All	0.225	0.1448	0.12	0.15

[a]Concentrations in water column, n=1.

[b]Mean values for 2003 do not include Big Creek data.

Table 2.8. Biological, toxicological and chemical parameter results with resulting ETR for selected Clinch River tributaries for 2003 and 2004 sampling years.

			Biological		Toxicological		
Land use	**Tributary**	**Year**	**SCI[a]**	**HA[b]**	**CG[c]**	**DR[d]**	**ETR[e]**
A	North Fork CR	2003	85	50	1.46	100	78
		2004	71	63	0.84	92	81
A	South Fork CR	2003	77	70	0.94	84	68
		2004	57	68	0.27	73	60
A	Cavitts Creek	2003	65	80	0.44	59	57
		2004	66	62	0.10	120	73
A	Big Cedar Creek	2003	96	73	0.67	50	73
		2004	83	61	0.70	89	86
A	Big Spring	2003	82	77	1.01	47	79
		2004	40	61	0.85	90	51
F	Big Creek	2003	90	73	0.71	53	78
M		2004	76	77	0.76	95	91
M	Swords Creek	2003	69	64	0.31	108	51
		2004	60	71	0.72	76	76
M	Lick Creek	2003	51	60	1.37	79	56
		2004	61	72	0.62	103	82
M	Guest River	2003	66	85	1.21	45	63
		2004	62	78	0.68	74	79
F	Big Stony Creek	2003	94	90	0.53	134	91
		2004	83	85	0.27	95	93
Agricultural		2003	81	70	0.90	68	71
		2004	63	63	0.55	93	70
Mining[f]		2003	62	70	0.96	77	57
		2004	65	75	0.70	87	82
Agricultural		All	72	67	0.73	80	71
Mining[f]		All	64	72	0.83	82	69

[a]Stream Condition Index
[b]Habitat assessment (%)
[c]Clam growth (mm)
[d]*Daphnia* reproduction (x)
[e]Ecotoxicological Rating
[f]Mean values for 2003 do not include Big Creek data

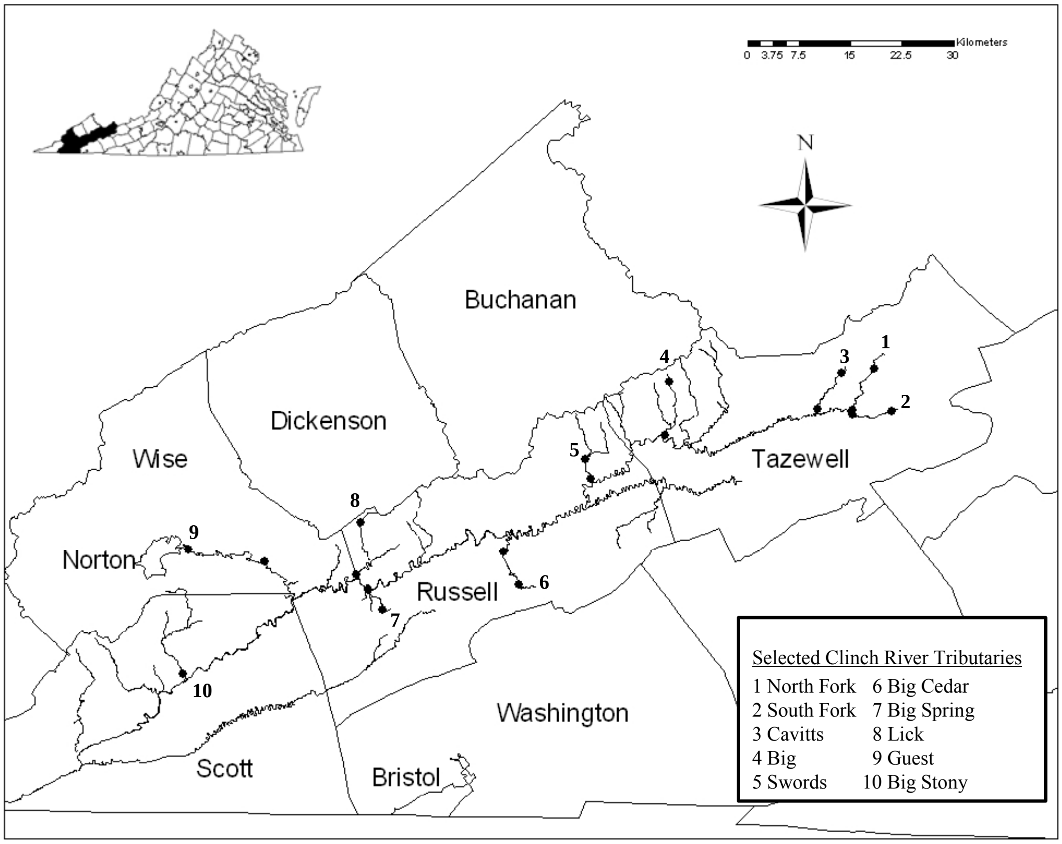

Figure 2.1. Selected Clinch River tributaries, upstream and downstream sites, sampled in 2004.

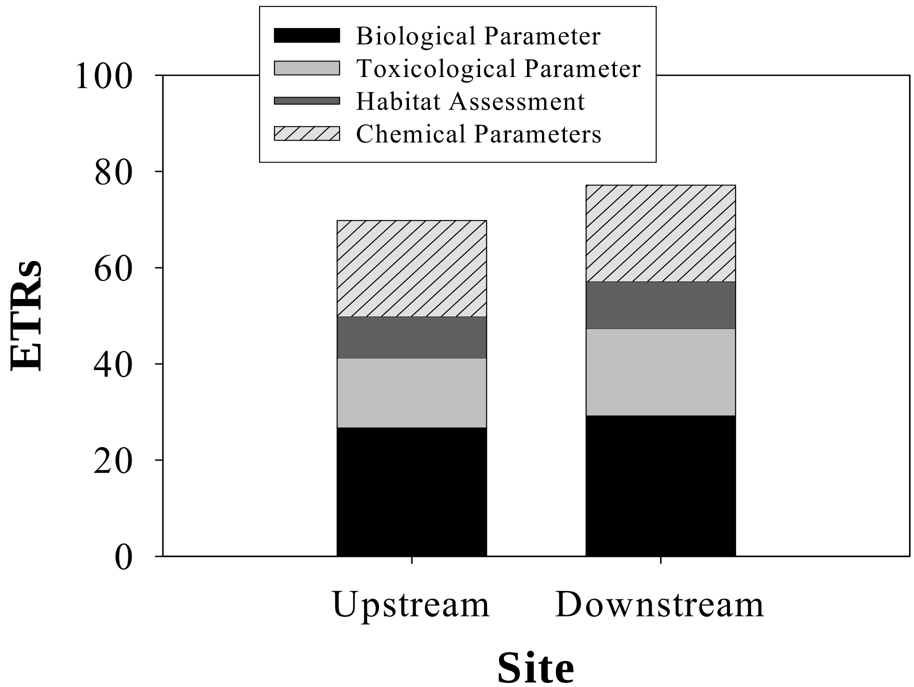

Figure 2.2. Ecotoxicological Ratings (ETRs) by site for Clinch River tributaries, Virginia.

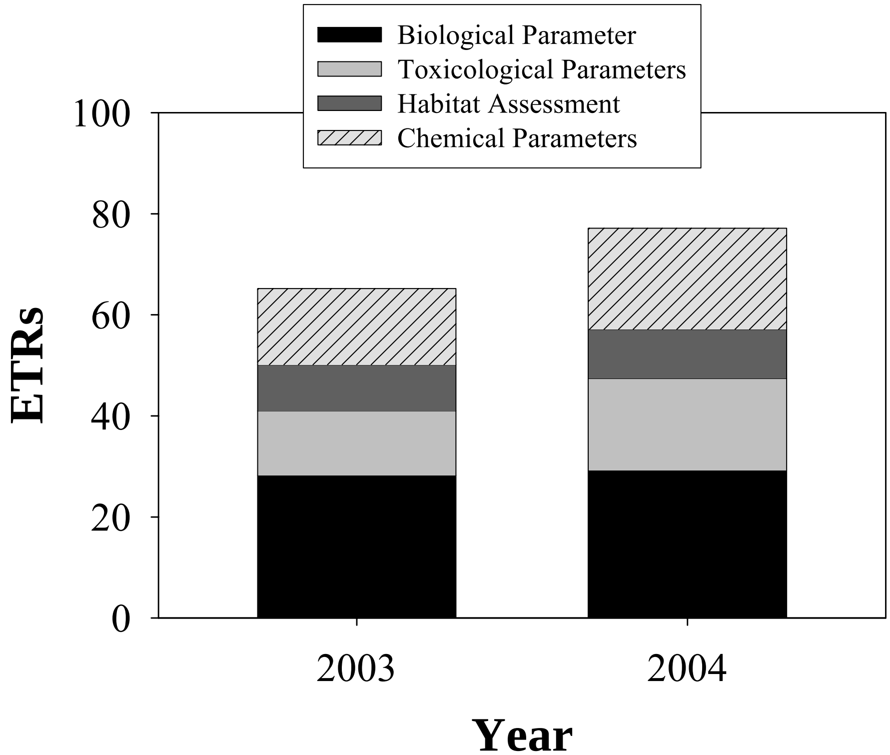

Figure 2.3. Ecotoxicological Ratings (ETRs) by year for Clinch River tributaries, Virginia.

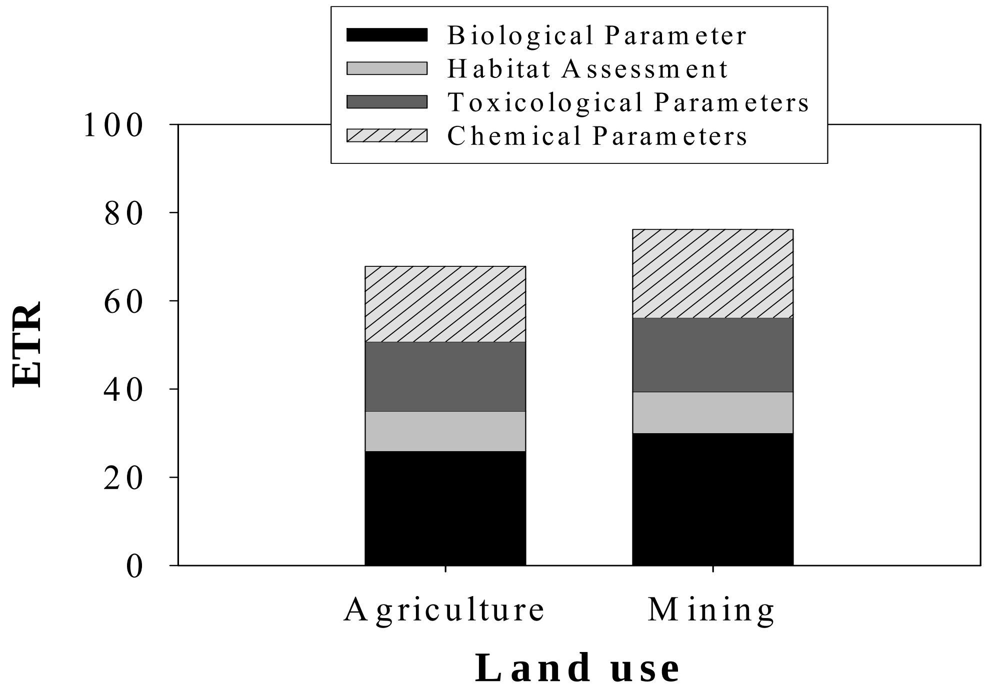

Figure 2.4. Ecotoxicological Ratings (ETRs) by land uses (A = agricultural and M = mining) of selected Clinch River tributaries, Virginia, 2004.

Appendix

The following pages are summaries for each studied Clinch River tributary including measurements that were not included in the previous chapters for the purpose of future reference. For the subsequent pages refer to the following tables. For analyzed metals, < (value) indicates below detection limit.

Table A.1. Acronyms and sampling sizes for additional variables.

ETR	Ecotoxicological Rating	
SCI	Stream Condition Index: US EPA (Developed by Tetra Tech) 2000	
%EPT-Hydro	Ephemeroptera, Plecoptera, Tricoptera (sensitive taxa) minus Hydrosycidae (tolerant taxa)	
HBI	Hilsenhoff Biotic Index: A pollution tolerant Index: Hilsenhoff 1977	
NO_3^-- N	Nitrate-N	n = 1
TP	Total Phosphorous	n = 1
SO_4^{2-}	Sulfate	n = 1
Al	Aluminum	n = 1
Mn	Manganese	n = 1
Fe	Iron	n = 1
Zn	Zinc	n = 1
Cu	Copper	n = 1
TDS	Total Dissolved Solids	n = 1
TSS	Total Suspended Solids	n = 1
Conductivity		n = 7 (2003) / n = 5 (2004)
pH		n = 7 (2003) / n = 5 (2004)
Alkalinity		n = 7 (2003) / n = 5 (2004)
Hardness		n = 7 (2003) / n = 5 (2004)

Table A.2. Stream Condition Index (SCI) score ratings described by Tetra Tech (US EPA 2000).

SCI Score	**Rating**
79-100	Comparable to reference site.
69-78	Comparable to or below average of reference site.
0-68	Increasingly different from reference site.

Table A.3. Water Quality Criteria for the following metals. The table includes chronic and acute values that indicate concentrations harmful to aquatic life, according to US EPA National Recommended Water Quality Criteria: 2002. EPA-822-R-02-047.

	Water Quality Criteria	
Metal	**Chronic value (mg/L)**	**Acute value (mg/L)**
Al	0.087	0.750
Mn	none	none
Fe	1.000	1.000
Zn	0.120	0.120
Cu	0.009	0.013

Table A.4. Recommended Water Quality Criteria values for the following nutrient levels. Values indicate the minimum, maximum, mean, median and 25% percentile of >200 sampled streams (all seasons) for Ecoregion XI: 67. US EPA Ambient Water Quality Criteria Recommendations for Rivers and Streams in Nutrient Ecoregion XI. EPA 822-B-00-020. 2002.

	Recommended Water Quality Criteria (mg/L)				
Nutrient	**Min**	**Max**	**Mean**	**Median**	**25%**
NO_3-N	0.003	5.960	0.693	0.440	0.230
TP	0.000	1.388	0.062	0.023	0.010
SO_4^{2-}	none	none	none	none	none

North Fork Clinch River

As part of the headwaters of the Clinch River, the North Fork originates northeast of Tazewell County and flows through the town of Tazewell. This watershed is dominated by forested land (54.5%) and agricultural land (43.2%), while 2% of the drainage is developed. Shells of dead mussels can be found at the downstream sampling site.

	North Fork Clinch River		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	78	81	81
SCI	85	71	94
Abundance	137	328	234
Richness	21	19	26
% Mayfly	51	39	40
%EPT-Hydro	54	43	53
%Chironomidae	18	31	21
HBI	4.889	4.503	4.415
NO_3-N (mg/L)	0.18	0.18	0.13
TP (mg/L)	0.11	0.17	0.22
SO_4^{2-} (mg/L)	3	1	2
Al (mg/L)	0.0827	0.0921	0.0389
Mn (mg/L)	0.0208	0.0572	0.0280
Fe (mg/L)	0.1176	0.2728	0.1393
Zn (mg/L)	<0.0050	0.0175	0.0098
Cu (mg/L)	<0.0061	<0.0015	0.0016
TDS (g/L)	0.117	0.217	0.200
TSS (g/L)	0.001	0.017	0.017
Conductivity (uS)	301	292	328
pH	8.34	7.92	7.97
Alkalinity (mg $CaCO_3$/L)	147	149	170
Hardness (mg $CaCO_3$/L)	156	158	186

South Fork Clinch River

The South Fork converges with the North Fork to form the headwaters of the Clinch River just north of the town of Tazewell, Tazewell County. The watershed is dominated by forested land (54%) and agricultural land (44%), while developed land is 1.6% of the drainage. Runoff from agricultural land and highway construction has caused siltation near the confluence of the Clinch River. Freshwater mussel shells can be found at the downstream sampling site.

	South Fork Clinch River		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	68	60	76
SCI	77	57	79
Abundance	117	149	251
Richness	23	14	26
% Mayfly	22	12	31
%EPT-Hydro	27	12	39
%Chironomidae	37	37	2
HBI	5.512	4.91	2.731
NO_3-N (mg/L)	0.23	0.23	0.26
TP (mg/L)	0.09	0.27	0.20
SO_4^{2-} (mg/L)	2	1	0
Al (mg/L)	0.0891	0.0609	0.0634
Mn (mg/L)	0.0112	0.0240	0.0519
Fe (mg/L)	0.0748	0.1018	0.1842
Zn (mg/L)	0.0074	0.0068	0.0523
Cu (mg/L)	<0.0061	<0.0015	<0.0015
TDS (g/L)	0.100	0.200	0.117
TSS (g/L)	0.001	0.010	0.027
Conductivity (uS)	287	280	284
pH	8.25	7.81	7.80
Alkalinity (mg $CaCO_3$/L)	139	138	134
Hardness (mg $CaCO_3$/L)	149	144	144

Cavitts Creek

Cavitts Creek flows into the Clinch River at River Jack, Tazewell County. The headwaters of Cavitts Creek originate from a lake drainage. Less than a mile downstream from the headwaters the creek runs behind a trailer park and then adjacent to a petroleum storage facility. Cavitts continues to flow south bordering Route 645 and behind a number of houses. The creek then passes a recycling dump, between a number of houses again before converging with the Clinch River. Cavitts' drainage consists of 64% forest, 34% agriculture, 1% developed and 55% other (predominately the lake).

	Cavitts Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	57	73	61
SCI	65	66	46
Abundance	106	162	33
Richness	17	16	10
% Mayfly	12	27	4
%EPT-Hydro	13	28	6
%Chironomidae	29	29	65
HBI	4.970	4.656	5.906
NO_3-N (mg/L)	0.16	0.26	0.10
TP (mg/L)	0.08	0.16	0.10
SO_4^{2-} (mg/L)	11	12	22
Al (mg/L)	0.0593	0.0549	0.0281
Mn (mg/L)	0.0172	0.0474	0.0536
Fe (mg/L)	0.0968	0.1927	0.1023
Zn (mg/L)	<0.0050	0.0048	0.0038
Cu (mg/L)	<0.0061	<0.0015	<0.0015
TDS (g/L)	0.217	0.250	0.183
TSS (g/L)	0.000	0.010	0.013
Conductivity (uS)	316	288	179
pH	8.24	7.88	7.97
Alkalinity (mg $CaCO_3$/L)	150	145	111
Hardness (mg $CaCO_3$/L)	159	154	93

Indian Creek

Indian Creek converges with the Clinch River at Cedar Bluff, Tazewell County. Active and abandoned coal mining can be found in the upper reaches of Indian's watershed. There are populations of three endangered mussel species (*Villosa perpurpurea, Epioblasma florentina walkeri, Quadrula cylindrica strigillata*) found in this tributary. Shells from dead mussels can be found at the downstream sampling site. Further studies were not conducted in this creek because of the endangered mussel assemblages and the fact that the 2003 ETR was >80%. Indian Creek's drainage consists of 89% forest, 9.5% agriculture, <1% developed and 0.8% mining. The creek does flow between several houses with straight pipes near the bottom of the watershed before meeting the Clinch River.

	Indian Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	86	N/A	N/A
SCI	81	N/A	N/A
Abundance	121	N/A	N/A
Richness	24	N/A	N/A
% Mayfly	27	N/A	N/A
%EPT-Hydro	37	N/A	N/A
%Chironomidae	33	N/A	N/A
HBI	4.841	N/A	N/A
NO_3-N (mg/L)	0.08	N/A	N/A
TP (mg/L)	0.09	N/A	N/A
SO_4^{2-} (mg/L)	23	N/A	N/A
Al (mg/L)	0.0845	N/A	N/A
Mn (mg/L)	0.0103	N/A	N/A
Fe (mg/L)	0.1540	N/A	N/A
Zn (mg/L)	0.0076	N/A	N/A
Cu (mg/L)	<0.0061	N/A	N/A
TDS (g/L)	0.067	N/A	N/A
TSS (g/L)	0.001	N/A	N/A
Conductivity (uS)	224	N/A	N/A
pH	8.29	N/A	N/A
Alkalinity (mg $CaCO_3$/L)	86	N/A	N/A
Hardness (mg $CaCO_3$/L)	103	N/A	N/A

Middle Creek

Like Indian Creek, Middle Creek joins the Clinch River at Cedar Bluff, Tazewell County. The drainage of Middle Creek is recovering from a reclaimed coal-processing site and hollow fill seeps. Merricks (2003) concluded that the most impaired reach of the creek was a drainage site of a hollow fill, approximately halfway downstream from the headwaters. Middle creek was not included in further studies since the entire catchment has been previously studied. This drainage is dominated by forest (97%), with 1% agriculture, <1% developed, and 1% mining. For further information on the status of Middle Creek see Merricks' thesis (2003).

	Middle Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	64	N/A	N/A
SCI	79	N/A	N/A
Abundance	24	N/A	N/A
Richness	13	N/A	N/A
% Mayfly	56	N/A	N/A
%EPT-Hydro	63	N/A	N/A
%Chironomidae	17	N/A	N/A
HBI	5.412	N/A	N/A
NO_3-N (mg/L)	0.08	N/A	N/A
TP (mg/L)	0.12	N/A	N/A
SO_4^{2-} (mg/L)	120	N/A	N/A
Al (mg/L)	0.0822	N/A	N/A
Mn (mg/L)	0.0668	N/A	N/A
Fe (mg/L)	0.2292	N/A	N/A
Zn (mg/L)	<0.0050	N/A	N/A
Cu (mg/L)	<0.0061	N/A	N/A
TDS (g/L)	0.150	N/A	N/A
TSS (g/L)	0.000	N/A	N/A
Conductivity (uS)	464	N/A	N/A
pH	8.22	N/A	N/A
Alkalinity (mg $CaCO_3$/L)	137	N/A	N/A
Hardness (mg $CaCO_3$/L)	149	N/A	N/A

Big Creek

Big Creek joins the Clinch River in Richlands, Tazewell County. The upper reach of Big Creek is reclaimed mining land. After the completion of conducted studies, AMD drainage, from the adjacent ridge, was present at the upstream sampling site. Traveling south, the creek flows through a more recently reclaimed area that had been reopened for active mining after the completion of the conducted studies. From this point, Big Creek continues flowing south adjacent to several houses. Runoff from reclaimed mining land and from houses is evident during rain events. Near the downstream sampling site there is waste water treatment plant. At the downstream sampling site there is a large metal waste water storage container that leaks, and possible overflows, during heavy rain events. These issues were especially apparent during the summer floods of 2004. The downstream sampling site has substantial quantities of dead mussel shells. The drainage is dominated by forest (96%), although agriculture (1.5%), developed (1%) and mining (1.6%) is also present.

	Big Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	78	91	84
SCI	90	76	81
Abundance	123	119	77
Richness	24	17	20
% Mayfly	37	23	23
%EPT-Hydro	49	23	42
%Chironomidae	20	9	24
HBI	4.270	4.525	4.736
NO_3-N (mg/L)	0.16	0.16	0.05
TP (mg/L)	0.13	0.13	0.13
SO_4^{2-} (mg/L)	23	25	49
Al (mg/L)	0.0628	0.0790	0.2999
Mn (mg/L)	0.0107	0.0227	0.2565
Fe (mg/L)	0.0907	0.1411	0.6280
Zn (mg/L)	0.0066	0.0039	0.0156
Cu (mg/L)	<0.0061	<0.0015	0.0040
TDS (g/L)	0.150	0.183	0.300
TSS (g/L)	0.002	0.003	0.003
Conductivity (uS)	322	308	338
pH	8.31	8.04	7.45
Alkalinity ($mgCaCO_3$/L)	136	148	91
Hardness ($mgCaCO_3$/L)	157	153	127

Coal Creek

Coal Creek converges with the Clinch River in Raven, Tazewell County. Active and abandoned mining are present in the upper reaches of the watershed as well as a coal-processing center and a coal slurry/sediment pond. Presently there are four existing permits. Runoff from railroad tracks as well as from houses adjacent to the creek also influences the watershed. The banks of the stream at the downstream sampling site are dominated by anthropogenic trash. Trash is also overwhelmingly present within the stream. Houses along the creek are dominated with straight pipe septic systems. Inaccessibility and personal safety hindered further studies in the upper portion of this catchment. The Coal Creek watershed is 88% forest land, 7% agriculture, approximately 2% developed and 3.7% mining. According to this study, Coal Creek is one of the most negatively impacted subwatersheds of the tributaries studied.

	Coal Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	48	N/A	N/A
SCI	51	N/A	N/A
Abundance	32	N/A	N/A
Richness	12	N/A	N/A
% Mayfly	21	N/A	N/A
%EPT-Hydro	22	N/A	N/A
%Chironomidae	51	N/A	N/A
HBI	5.912	N/A	N/A
NO_3-N (mg/L)	0.08	N/A	N/A
TP (mg/L)	0.08	N/A	N/A
SO_4^{2-} (mg/L)	63	N/A	N/A
Al (mg/L)	<0.0525	N/A	N/A
Mn (mg/L)	0.0958	N/A	N/A
Fe (mg/L)	0.1021	N/A	N/A
Zn (mg/L)	0.0064	N/A	N/A
Cu (mg/L)	<0.0061	N/A	N/A
TDS (g/L)	0.350	N/A	N/A
TSS (g/L)	0.001	N/A	N/A
Conductivity (uS)	616	N/A	N/A
pH	8.18	N/A	N/A
Alkalinity (mg $CaCO_3$/L)	107	N/A	N/A
Hardness (mg $CaCO_3$/L)	158	N/A	N/A

Swords Creek

Swords Creek flows into the Clinch River at the junction of Route 67 and 633, north of Gardner, Tazewell County. The catchment is 91% forest, 5% agriculture, <1% developed and nearly 3% mining influenced land. Active and abandoned mining are present. Runoff from these influences as well as from a masonry plant and houses are present. Mudslides were prevalent, houses were lost and roads were damaged during the summer floods of 2004.

	Swords Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	51	76	84
SCI	69	60	79
Abundance	107	41	22
Richness	19	11	14
% Mayfly	50	51	36
%EPT-Hydro	54	52	38
%Chironomidae	33	11	10
HBI	5.190	5.392	5.259
NO_3-N (mg/L)	0.13	0.10	0.10
TP (mg/L)	0.18	0.15	0.12
SO_4^{2-} (mg/L)	56	66	32
Al (mg/L)	0.0940	0.6920	0.2217
Mn (mg/L)	0.0182	0.3614	0.0978
Fe (mg/L)	0.1276	1.976	0.6960
Zn (mg/L)	0.0127	0.0278	0.0143
Cu (mg/L)	<0.0061	0.0038	<0.0015
TDS (g/L)	0.167	0.133	0.200
TSS (g/L)	0.001	0.013	0.057
Conductivity (uS)	256	243	157
pH	8.09	7.83	7.76
Alkalinity (mg $CaCO_3$/L)	73	73	56
Hardness (mg $CaCO_3$/L)	117	108	59

Little River

Little River joins the Clinch River at the junction of Route 641 and 640 in Russell County. The drainage is of the valley portion of the Ridge and Valley and is comprised of 55% forest, 45% agriculture, and <1% developed and mining influenced land. Several houses line the stream traveling upstream. Dead mussel shells are prevalent at the confluence with the Clinch River. Runoff is evident during rain events.

	Little River		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	82	N/A	N/A
SCI	96	N/A	N/A
Abundance	98	N/A	N/A
Richness	25	N/A	N/A
% Mayfly	33	N/A	N/A
%EPT-Hydro	44	N/A	N/A
%Chironomidae	17	N/A	N/A
HBI	4.323	N/A	N/A
NO_3-N (mg/L)	0.21	N/A	N/A
TP (mg/L)	0.13	N/A	N/A
SO_4^{2-} (mg/L)	7	N/A	N/A
Al (mg/L)	0.0541	N/A	N/A
Mn (mg/L)	0.0033	N/A	N/A
Fe (mg/L)	0.0496	N/A	N/A
Zn (mg/L)	0.0054	N/A	N/A
Cu (mg/L)	<0.0061	N/A	N/A
TDS (g/L)	0.150	N/A	N/A
TSS (g/L)	0.000	N/A	N/A
Conductivity (uS)	269	N/A	N/A
pH	8.42	N/A	N/A
Alkalinity (mg $CaCO_3$/L)	133	N/A	N/A
Hardness (mg $CaCO_3$/L)	147	N/A	N/A

Big Cedar Creek

Big Cedar Creek meets the Clinch River at Big Falls, Russell County. This point is inaccessible via road. A tributary to Big Cedar, Little Cedar Creek, flows through the town of Lebanon before joining Big Cedar Creek. Just past this junction is a waste water treatment plant, between the upstream and downstream sampling sites. The drainage was previously a pasture/livestock area and is presently mostly hay fields. The catchment is on the valley side of the Ridge and Valley and encompasses 71% forest land, 28% agriculture, and 1% developed. If revisiting, beware of snakes.

	Big Cedar Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	73	81	86
SCI	96	88	83
Abundance	166	211	185
Richness	30	24	25
% Mayfly	39	50	45
%EPT-Hydro	48	50	52
%Chironomidae	20	19	10
HBI	4.711	4.605	4.659
NO_3-N (mg/L)	0.23	0.34	0.26
TP (mg/L)	0.16	0.15	0.11
SO_4^{2-} (mg/L)	12	5	1
Al (mg/L)	0.0775	0.1534	0.0715
Mn (mg/L)	0.0077	0.0655	0.0330
Fe (mg/L)	0.0746	0.2260	0.1169
Zn (mg/L)	<0.0050	0.0089	0.0106
Cu (mg/L)	<0.0061	<0.0015	<0.0015
TDS (g/L)	0.167	0.117	0.183
TSS (g/L)	0.001	0.003	0.013
Conductivity (uS)	317	332	294
pH	8.27	8.28	8.2
Alkalinity (mg$CaCO_3$/L)	157	173	172
Hardness (mg$CaCO_3$/L)	173	178	172

Dumps Creek

Dumps Creek's confluence with the Clinch River is at Carbo, Russell County. American Electric Company's (AEP) plant is directly across from the junction of Dumps Creek and the Clinch River. Dumps Creek is influenced by active and abandoned mining, mine-waste disposal sites and a coal processing plant. Sedimentation is overwhelming at it's confluence and is apparent in upper reaches of the watershed. The drainage is 91% forest, 1.5% agriculture, nearly 7% mining and <1% developed land. This study indicated Dumps Creek is one of the most negatively impacted subwatersheds of those studied. See Hull's thesis (2002) for further status on the entire catchment.

	Dumps Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	44	N/A	N/A
SCI	54	N/A	N/A
Abundance	13	N/A	N/A
Richness	9	N/A	N/A
% Mayfly	7	N/A	N/A
%EPT-Hydro	16	N/A	N/A
%Chironomidae	34	N/A	N/A
HBI	4.914	N/A	N/A
NO_3-N (mg/L)	0.10	N/A	N/A
TP (mg/L)	0.12	N/A	N/A
SO_4^{2-} (mg/L)	120	N/A	N/A
Al (mg/L)	0.1715	N/A	N/A
Mn (mg/L)	0.0401	N/A	N/A
Fe (mg/L)	0.1572	N/A	N/A
Zn (mg/L)	<0.0050	N/A	N/A
Cu (mg/L)	<0.0061	N/A	N/A
TDS (g/L)	0.333	N/A	N/A
TSS (g/L)	0.003	N/A	N/A
Conductivity (uS)	574	N/A	N/A
pH	8.30	N/A	N/A
Alkalinity (mg $CaCO_3$/L)	191	N/A	N/A
Hardness (mg $CaCO_3$/L)	149	N/A	N/A

Big Spring

Big Spring flows into the Clinch River west of Castlewood, Russell County. The catchment is heavily influenced by a large trailer park with straight pipe septic systems. Eutrophication is prevalent at the downstream sampling site, downstream of the trailer park. Big Spring is what it's name says, a big spring, and therefore macroinvertebrate populations are going to be different than other studied tributaries. More precise bug sampling techniques during 2004 could have explained variation in bug metrics between years, thus explaining variation in ETRs between years. Watershed area is 65% forest, 33% agriculture, and almost 3% developed. This tributary is on the valley side of the Ridge and Valley and is a smaller drainage than others included in this study.

	Big Spring		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	79	51	41
SCI	82	40	39
Abundance	171	188	220
Richness	24	13	15
% Mayfly	35	5	0
%EPT-Hydro	37	6	0
%Chironomidae	19	82	63
HBI	4.727	5.719	6.336
NO_3-N (mg/L)	0.23	0.26	0.34
TP (mg/L)	0.10	0.11	0.12
SO_4^{2-} (mg/L)	25	39	47
Al (mg/L)	<0.0525	0.0388	0.0313
Mn (mg/L)	0.0136	0.0126	0.0072
Fe (mg/L)	0.0624	0.0543	0.0398
Zn (mg/L)	0.0052	0.0114	0.0055
Cu (mg/L)	<0.0061	0.0016	<0.0015
TDS (g/L)	0.233	0.217	0.267
TSS (g/L)	0.002	0.033	0.000
Conductivity (uS)	417	384	439
pH	8.32	8.37	7.94
Alkalinity (mg$CaCO_3$/L)	185	204	226
Hardness (mg$CaCO_3$/L)	233	231	242

Lick Creek

Lick Creek and the Clinch River intersect at Saint Paul on the border of Russell and Wise County. Active and abandoned mining are present, as well as a coal-processing site and a holding pond at the bottom of the watershed adjacent to the downstream sampling site. A waste water treatment plant, recently built, can be found approximately 1.5 miles upstream. Traveling upstream, houses and railroad tracks border the stream. Several railroad tracks suggest heavy coal extraction at one time. Lick Creek has historically been said by local residents to "run black." The drainage is defined by 93% forest, 5% agriculture, 1% developed and almost 2% mining.

	Lick Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	56	82	41
SCI	51	61	40
Abundance	63	152	72
Richness	13	12	12
% Mayfly	24	22	13
%EPT-Hydro	25	24	14
%Chironomidae	53	38	68
HBI	5.710	5.967	5.766
NO_3-N (mg/L)	0.08	0.08	0.13
TP (mg/L)	0.11	0.12	0.24
SO_4^{2-} (mg/L)	124	100	136
Al (mg/L)	<0.0525	0.0395	0.0334
Mn (mg/L)	0.0196	0.0192	0.015
Fe (mg/L)	0.0821	0.1291	0.0961
Zn (mg/L)	<0.0050	0.0035	0.0085
Cu (mg/L)	<0.0061	<0.0015	<0.0015
TDS (g/L)	0.300	0.267	0.317
TSS (g/L)	0.001	0.007	0.007
Conductivity (uS)	478	451	603
pH	8.32	8.54	8.31
Alkalinity (mg $CaCO_3$/L)	112	126	216
Hardness (mg $CaCO_3$/L)	182	188	215

Russell Creek

Russell Creek converges with the Clinch River via waterfall at Clinchfield, Wise County. Active and abandoned mining are present (three permits on Russell Creek and two permits on a tributary to Russell Creek). There is a holding pond at the toe of a hollow fill upstream from the downstream sampling site. Forest land describes 89% of the subwatershed, almost 4% agriculture and just over 7% by mining. Further studies could not be conducted on the upper reaches of the watershed because of inaccessibility due to permits for coal mining. This study indicates Russell Creek is one of the most negatively influenced tributaries of those studied.

	Russell Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	46	N/A	N/A
SCI	58	N/A	N/A
Abundance	57	N/A	N/A
Richness	9	N/A	N/A
% Mayfly	14	N/A	N/A
%EPT-Hydro	14	N/A	N/A
%Chironomidae	15	N/A	N/A
HBI	6.030	N/A	N/A
NO_3-N (mg/L)	0.05	N/A	N/A
TP (mg/L)	0.10	N/A	N/A
SO_4^{2-} (mg/L)	2200	N/A	N/A
Al (mg/L)	0.1253	N/A	N/A
Mn (mg/L)	0.1171	N/A	N/A
Fe (mg/L)	0.4233	N/A	N/A
Zn (mg/L)	<0.0050	N/A	N/A
Cu (mg/L)	<0.0061	N/A	N/A
TDS (g/L)	0.500	N/A	N/A
TSS (g/L)	0.001	N/A	N/A
Conductivity (uS)	859	N/A	N/A
pH	8.10	N/A	N/A
Alkalinity (mg $CaCO_3$/L)	246	N/A	N/A
Hardness (mg $CaCO_3$/L)	211	N/A	N/A

Guest River

The Guest River (Wise County) is the largest tributary feeding the Clinch River. It flows past Norton, through Coeburn and through the Jefferson National Forest before it's confluence with the Clinch River at the Wise, Scott and Russell County boundaries. Active and abandoned mining are present, as well as AMD and coal storage piles. It also flows through a coal housing facility in Norton. Coal fines are prevalent at both sampling sites. The largest percentage of watershed area of all studied tributaries is described by mining (9%), while 79% is defined by forest and roughly 6% by agriculture and developed land. Trash in the creek bed is apparent at both sampling sites, especially the upstream site where trash is evident in and along the stream banks as well. Previous studies have been conducted on the Guest River and in 1995 the Guest River Group, an alliance formed to protect and restore the watershed, began restoring the catchment. The elimination of straight pipes and the pumpout of 400 residential septic tanks have attributed to the improvement of the Guest River.

	Guest River		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	63	79	73
SCI	66	62	59
Abundance	54	176	33
Richness	16	9	12
% Mayfly	18	38	8
%EPT-Hydro	20	38	17
%Chironomidae	34	17	17
HBI	5.646	6.057	5.428
NO_3-N (mg/L)	0.10	0.16	0.16
TP (mg/L)	0.19	0.17	0.14
SO_4^{2-} (mg/L)	1900	130	400
Al (mg/L)	0.0643	0.0663	0.0259
Mn (mg/L)	0.1517	0.0812	0.0993
Fe (mg/L)	0.2195	0.2411	0.2648
Zn (mg/L)	0.0066	0.0126	0.0036
Cu (mg/L)	<0.0061	<0.0015	<0.0015
TDS (g/L)	0.283	0.217	0.533
TSS (g/L)	0.001	0.020	0.007
Conductivity (uS)	507	545	862
pH	8.03	8.28	8.02
Alkalinity (mg $CaCO_3$/L)	100	110	127
Hardness (mg $CaCO_3$/L)	208	192	148

Big Stony Creek

Big Stony Creek meets the Clinch River just south of Fort Blackmore, Scott County, due east of Pendleton Island where freshwater mussel richness is vast. This tributary flows through the Jefferson National Forest and served as the reference stream throughout the conducted studies. A strawberry patch, which draws water from Big Stony, is adjacent to the creek just upstream from the confluence with the Clinch River. Forest land is >98% of the watershed area and just over 1% is agriculture. An upstream site was not assessed due to inaccessibility.

	Big Stony Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	91	93	N/A
SCI	94	83	N/A
Abundance	103	122	N/A
Richness	23	25	N/A
% Mayfly	66	29	N/A
%EPT-Hydro	77	40	N/A
%Chironomidae	16	20	N/A
HBI	4.144	4.475	N/A
NO_3-N (mg/L)	0.08	0.08	N/A
TP (mg/L)	0.11	0.16	N/A
SO_4^{2-} (mg/L)	3	3	N/A
Al (mg/L)	0.0588	0.0235	N/A
Mn (mg/L)	0.0172	0.0173	N/A
Fe (mg/L)	0.0333	0.0433	N/A
Zn (mg/L)	<0.0050	0.0134	N/A
Cu (mg/L)	<0.0061	0.0016	N/A
TDS (g/L)	0.050	0.050	N/A
TSS (g/L)	0.001	0.000	N/A
Conductivity (uS)	97	115	N/A
pH	7.76	8.13	N/A
Alkalinity (mg$CaCO_3$/L)	47	63	N/A
Hardness (mg$CaCO_3$/L)	50	60	N/A

Cove Creek

Cove Creek flows into the Clinch River southwest of Kerns and north of Slant, Scott County. It flows through the Jefferson National Forest and is 88% forest and 12% agriculture. There is active livestock grazing neighboring the sampling site and the bridge at the sampling site has caused siltation. Cove Creek was deemed healthy and not studied further despite having an ETR <80% because it was concluded that it's score would have been higher had benthic macroinvertebrate sampling taken place upstream or downstream of the bridge influence.

	Cove Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	79	N/A	N/A
SCI	84	N/A	N/A
Abundance	42	N/A	N/A
Richness	19	N/A	N/A
% Mayfly	58	N/A	N/A
%EPT-Hydro	60	N/A	N/A
%Chironomidae	7	N/A	N/A
HBI	4.225	N/A	N/A
NO_3-N (mg/L)	0.13	N/A	N/A
TP (mg/L)	0.08	N/A	N/A
SO_4^{2-} (mg/L)	11	N/A	N/A
Al (mg/L)	0.0691	N/A	N/A
Mn (mg/L)	0.0074	N/A	N/A
Fe (mg/L)	0.0446	N/A	N/A
Zn (mg/L)	<0.0050	N/A	N/A
Cu (mg/L)	<0.0061	N/A	N/A
TDS (g/L)	0.117	N/A	N/A
TSS (g/L)	0.000	N/A	N/A
Conductivity (uS)	250	N/A	N/A
pH	8.05	N/A	N/A
Alkalinity (mg $CaCO_3$/L)	119	N/A	N/A
Hardness (mg $CaCO_3$/L)	137	N/A	N/A

Stock Creek

Stock Creek merges with the Clinch River at Clinchport, Scott County. It drains the Jefferson National Forest. A closed lithium mine is upstream and houses border the stream but do not influence the health of it. The catchment is 96% forest land and just over 3% agriculture land.

	Stock Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	84	N/A	N/A
SCI	84	N/A	N/A
Abundance	39	N/A	N/A
Richness	15	N/A	N/A
% Mayfly	45	N/A	N/A
%EPT-Hydro	50	N/A	N/A
%Chironomidae	9	N/A	N/A
HBI	4.280	N/A	N/A
NO_3-N (mg/L)	0.08	N/A	N/A
TP (mg/L)	0.03	N/A	N/A
SO_4^{2-} (mg/L)	13	N/A	N/A
Al (mg/L)	<0.0525	N/A	N/A
Mn (mg/L)	0.0093	N/A	N/A
Fe (mg/L)	0.0583	N/A	N/A
Zn (mg/L)	<0.0050	N/A	N/A
Cu (mg/L)	<0.0061	N/A	N/A
TDS (g/L)	0.217	N/A	N/A
TSS (g/L)	0.001	N/A	N/A
Conductivity (uS)	246	N/A	N/A
pH	7.98	N/A	N/A
Alkalinity (mg $CaCO_3$/L)	103	N/A	N/A
Hardness (mg $CaCO_3$/L)	119	N/A	N/A

Copper Creek

Copper Creek flows into the Clinch River north of Speers Ferry, Scott County. Draining the valley side of the Ridge and Valley, it is described by 72% forest, 24% agriculture and <1% developed land. Agriculture dominates the landscape following the creek downstream. Freshwater mussel assemblages are known to exist in this catchment and live mussels are present at the upstream sampling site. Dead mussel shells are present at the downstream sampling site.

	Copper Creek		
Parameter	**2003**	**2004**	**upstream 2004**
ETR	86	N/A	N/A
SCI	88	N/A	N/A
Abundance	37	N/A	N/A
Richness	13	N/A	N/A
% Mayfly	42	N/A	N/A
%EPT-Hydro	57	N/A	N/A
%Chironomidae	1	N/A	N/A
HBI	3.498	N/A	N/A
NO_3-N (mg/L)	0.21	N/A	N/A
TP (mg/L)	0.06	N/A	N/A
SO_4^{2-} (mg/L)	11	N/A	N/A
Al (mg/L)	<0.0525	N/A	N/A
Mn (mg/L)	0.0047	N/A	N/A
Fe (mg/L)	0.0418	N/A	N/A
Zn (mg/L)	<0.0050	N/A	N/A
Cu (mg/L)	<0.0061	N/A	N/A
TDS (g/L)	0.267	N/A	N/A
TSS (g/L)	0.000	N/A	N/A
Conductivity (uS)	325	N/A	N/A
pH	8.12	N/A	N/A
Alkalinity (mg $CaCO_3$/L)	162	N/A	N/A
Hardness (mg $CaCO_3$/L)	213	N/A	N/A

Branden Alyssa Locke

Curriculum Vita

March 1, 2005

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713 South Main St
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Blacksburg, VA 24060
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EDUCATION:

Graduate

Virginia Polytechnic Institute and State University, Blacksburg VA.
Master's in Biology with emphasis in Aquatic Ecotoxicology (August 2002 – May 2005).
Thesis title: An Ecotoxicological Assessment of Major Tributaries to the Upper Clinch River, Virginia.

Undergraduate

Mary Washington College, Fredericksburg VA
Bachelor of Science Degree 2000
Biology with a double major in Geography

PROFESSIONALEXPERIENCE:

Graduate Teaching Assistant — **January 2005 – April 2005**

Virginia Tech, Department of Biology, Blacksburg, VA

- Laboratory instructor for Freshwater Ecology classes
- Guided / mentored / assisted student developed research projects

Graduate Research Assistant — **August 2002 – January 2005**

Cherry Lab, Virginia Tech, Department of Biology, Blacksburg, VA

- Conducted toxicity tests for National Pollutant Discharge & Elimination System Permits
- Culturing *Ceriodaphnia dubia* & *Daphnia magna* (all aspects)
- Conducted toxicity testing utilizing *C. dubia*, *D. magna* & *Pimephales promelas*
- Conducted sediment toxicity testing using D. magna
- Transplanted Asian clam (*Corbicula fluminea*) studies
- Benthic macroinvertebrates collection and identification (Qualitative/Quantitative)
- Formulated EPA^{100} moderately-hard reconstituted water
- Microbalances to measure dry weight of organisms
- Alkalinity/hardness titration
- Analyzed physicochemical parameters
- Performed habitat assessments (US EPA RBP)
- Assisted toxicity testing with early life-stage unionids

MS research: An assessment of 19 major tributaries as point-source influences to the Clinch River. Parameters used to determine aquatic health of each subwatershed included, water chemistry, acute and chronic water column toxicity testing, sediment toxicity testing, benthic macroinvertebrate surveys, and *in situ* studies using the Asian clam. An Ecotoxicological Rating, a method for combining multiple response variables into one numeric value from 1-100, was developed to rank tributaries from most to least healthy. Geographic Information Systems was used to characterize catchments by land use.

Graduate Teaching Assistant — **January 2004 – May 2004**

Virginia Tech, Department of Biology, Blacksburg, VA

- Laboratory instructor for General Biology classes
- Developed course syllabus and examinations

Graduate Teaching Assistant **August 2003/2004 – December 2003/2004**
Virginia Tech, Department of Biology, Blacksburg, VA

- Field laboratory instructor for Mammalogy classes
- Taught proper field methodology in trapping, tagging, identifying and collecting physical characteristics

Pharmacy Assistant **October 2001 – July 2002**
Virginia-Maryland Regional Veterinary Hospital, Blacksburg, VA

- Proofed vet students' calculations for accuracy and proper dosages of pharmaceuticals
- Responsible for compounding drugs, making antigens and preparing IV bags

Equine Veterinary Assistant **February 2001 – May 2001**
Equine Veterinary Associates, Portland, CT

- Responsible for assisting with the regular maintenance of horses including injections, artificial insemination, surgical procedures and diagnosis of sickness and lameness
- Developed radiographs and compiled blood samples for the laboratory

Barn Supervisor **May 1996 – June 2000**
Hazelwild Farm, Fredericksburg, VA

- Managed two barns, caring for 35 privately owned horsed including five broodmares
- Treated surface-level injuries, administered muscular shots, wormed horses, and was independently responsible for the safety and maintenance of animals
- Worked as many as 50 hours a week while a full time student

PUBLICATIONS:

Locke B.A., D.S. Cherry, C.E. Zipper, & R.J. Currie. 2005. Land Use Influences and Ecotoxicological Ratings for Upper Clinch River Tributaries, Virginia. (In review).

Valenti T.W., D.S. Cherry, R.J. Neves, & B.A. Locke. 2005. Sensitivity of glochidia and regulatory test organisms to mercury and a reference toxicant. (In review).

Valenti T.W., D.S. Cherry, R.J. Neves, B.A. Locke, J.M. Uerz, & R.J. Currie. 2003. Acute and chronic mercury exposures to different life stages of the rainbow mussel, Villosa iris. SETAC 24th Annual Meeting in North America. Austin, Texas. Poster presentation.

INVITED LECTURER:

Locke, B.A. Land Use Influences and Ectoxicological Ratings for Upper Clinch River Tributaries, Virginia. Presented at Roanoke College, Roanoke, Virginia on March 4, 2005.

TECHNICAL REPORTS:

Quarterly NPDES permit reports:
International Paper
Celanese Acetate
Acute and Chronic Biomonitoring with Ceriodaphnia dubia and Pimephales promelas

Cherry, D.S., R.J. Currie, T.W. Valenti, B.A. Locke, M.L Simon and B.S. Echols. 2004. Toxicity Test Laboratory Performance Evaluation (DMR-QA 24 WET) for Acute and Chronic Tests. US EPA Round Robin Evaluations.

Cherry, D.S., R.J. Currie, T.C. Merricks, T.W. Valenti, B.A. Locke. 2002-2004. Acute/Chronic Toxicity Evaluation of 001 Effluent Celanese Acetate, Celco Plant, Virginia.

Cherry, D.S., R.J. Currie, T.C. Merricks, T.W. Valenti, B.A. Locke. 2002-2004. Acute Toxicity Evaluation of 002 Effluent from Celanese Acetate, Celco Plant, Virginia.

Cherry, D.S., R.J. Currie, T.W. Valenti, B.A. Locke, J.M. Uerz. 2002-2004. Acute Toxicity Evaluation of 003 Effluent from Celanese Acetate, Celco Plant, Virginia.

Cherry, D.S., R.J. Currie, T.W. Valenti, B.A. Locke, J. M. Uerz. 2002-2004. Quarterly Chronic Biomonitoring Results of a Water Flea (Ceriodaphnia dubia) and Fathead Minnow (Pimephales promelas) to Pine Bluff Mill Effluent, International Paper Company, Pine Bluff, Arkansas.

ORGANIZATIONS:

Society of Environmental Toxicology and Chemistry 2003-2004
Appalachian Voices 2003-2004

COMPUTER SKILLS:

Microsoft office programs (Word, Excel, PowerPoint, FrontPage)
Webpage design and management
Toxstat
JMPIN
Limited use of SAS
Limited use of Geographical Information System – ArcView 8.3 and extensions

PROFESSIONAL REFERENCES:

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