LONG-TERM BIOASSESSMENT OF MINED VS. UN-MINED STREAMS IN THE CUMBERLAND MOUNTAINS OF EAST TENNESSEE BASED ON THIRTY YEARS OF COLLECTED DATA

Elizabeth D. Slade

LONG-TERM BIOASSESSMENT OF MINED VS. UN-MINED STREAMS IN THE CUMBERLAND MOUNTAINS OF EAST TENNESSEE BASED ON THIRTY YEARS OF COLLECTED DATA

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Master of Science Degree

Elizabeth D. Slade

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To the Graduate Council:

We are submitting a thesis written by Elizabeth D. Slade entitled "Long-term Bioassessment of Mined versus Un-Mined Streams in the Cumberland Mountains of East Tennessee Based on Thirty Years of Collected Data." We have examined the final copy of the thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

Joseph R. Schiller, Committee Chair

Steven W. Hamilton, Committee Member

Rebecca J. Johansen, Committee Member

Accepted for the Graduate and Research Council

Dr. Michael Gotcher
Interim Dean, College of Graduate Studies

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DEDICATION

I dedicate this thesis to the two men in my life who tried their best to keep me sane throughout this project. First, and foremost, I dedicate this thesis to my husband Kevin Slade. You were my rock, my shelter in the storm as I traveled this difficult path, always believing in me, always there with encouragement, even from a combat zone in Afghanistan. You never let me quit, especially during those times when I was ready to throw in the towel and walk away. You never questioned my ability to make it through, to triumph when all I saw was failure. You've sacrificed and supported me, and never complained when I spent all my time surrounded by textbooks, articles, and spreadsheets. For this, and so much more, I can never be thankful enough.

Second, but certainly not last, I dedicate this thesis to my brother Rick Haynes. I didn't have time to tell you these things before, so this is my tribute to you. I remember spending hours on the phone talking to you, sometimes about nothing at all. You had that special, wonderful ability to make me laugh when I wanted to cry. You always had time for me. You were everything a big brother should be, and more than any sister could ever hope for. Though you didn't live to see this project completed, you are with me in spirit; I feel your hand on my shoulder every day. May the earth rise up to meet you, and may the wind always be at your back. Namaste, my brother.

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major...I needed one more course to be full time and Aquatic Macro happened to fit my schedule. I had no idea how much life was in that yucky, stinky cattle pond; I was hooked with the first net. By the end of the semester, everyone in med tech was calling me “Grissom” or “bug lady”. I knew I had found my niche in biology. You have endured years of my endless “quick questions” and “I know you’re busy, but can you come look at this one?” with grace and the patience of a saint. You have my profound thanks.

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ABSTRACT

Elizabeth Dawn Slade. Long-term Bioassessment of Mined versus Un-Mined Streams in the Cumberland Mountains of East Tennessee Based on Thirty Years of Collected Data. (Under the direction of Dr. Joseph R. Schiller)

This study consisted of macroinvertebrate bioassessments of six streams in Campbell and Scott Counties of East Tennessee sampled in 2011 to bioassessments of four of the same streams sampled in 1979, 1980, 1981, and 2008 (Whitley, 2009; Vaughan et al., 1982). The studied streams vary in mining history and included two un-mined, two lightly mined 30 years before present, and two extensively mined streams. The mined streams also vary in that two were mined prior to implementation of federal reclamation regulations while two were mined both before and after the implementation of federal regulations.

Standard abiotic measurements of temperature, dissolved oxygen, pH and conductivity were recorded in the field using a portable YSI Environmental Monitoring System (650QS Display/Logger) and alkalinity and total hardness were titrated in the field using a Hach Kit (model number FF-1A).

All studies collected macroinvertebrates using Surber samplers, identified them to the lowest taxon possible (primarily to genus). This study conducted bioassessments on the macroinvertebrate collections of all the studies in accordance with the Tennessee Department of Environmental Conservation's (TDEC) Tennessee Macroinvertebrate Index (TDEC, 2006). The objectives of this study were to: 1) Conduct macroinvertebrate bioassessment of six streams in the Cumberland Mountains of East Tennessee; 2) Compare bioassessments among studies to assess: (a) the recovery of stream macroinvertebrate communities from strip mining disturbance, and (b) the effectiveness of current mine reclamation requirements; 3) Assess the effects of drought conditions on bioassessments conducted in 2008; and 4) Assess the variability of bioassessments on the same stream in the same season among years. Bioassessments over time indicate significant recovery of mined streams from strip mining impacts and seem to affirm the efficacy of federal regulations. The drought of 2008 did not have a significant negative impact on bioassessment classifications. Bioassessments among years on the same streams varied, including un-mined streams, indicating a need for repeated bioassessment to obtain reliable results.

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CHAPTER I

INTRODUCTION

STUDY AREA

The streams in this study are located in the Cumberland Mountains of East Tennessee. These mountains extend through three climatological divisions, depending on elevation; with an annual precipitation of approximately 50 inches and an average annual temperature of 56°F. The frost-free season consists of approximately 160 days per year, extending from late April through early October (USGS, 1982). The mountains rise to elevations of 2000-3000 feet above sea level and consist of steep terrain with slopes averaging 20-60% (U.S. Department of the Interior, Geological Survey). The forest type in this area is primarily mixed mesophytic; consisting of a diverse mix of conifers such as yellow and white pines, hemlock, and various hardwoods such as sugar maples, oaks, hickories, tulip poplars, etc. (TDEC, 2012). The streams in this study fall within the Tennessee Department of Environment and Conservation (TDEC) ecoregion 69D04 (TDEC, 2006) and are located in Campbell and Scott counties, Tennessee (Fig. 1.1). The six study streams are small headwater tributaries of the New River in Scott County (Lowe, Branch, Bowling Branch, Bills Branch and Green Branch) and the Clear Fork River in Campbell County, Tennessee (Crabapple Creek and Bruce Creek).

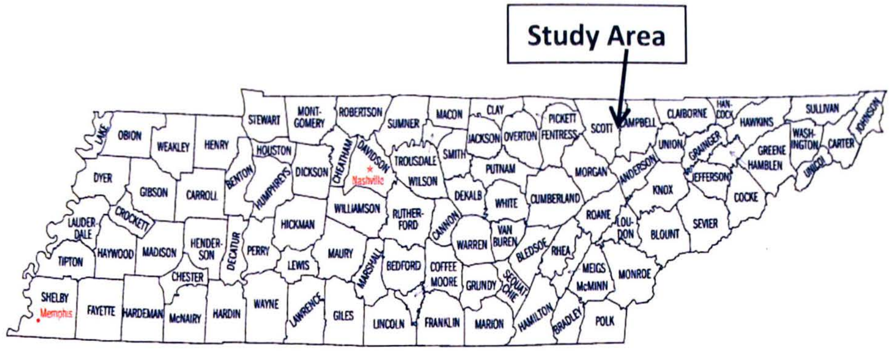


Figure 1.1. Map of Tennessee showing location of Scott and Campbell Counties.

Lowe Branch is a tributary to the New River, while Bowling Branch, Bills Branch, and Green Branch are tributaries of Smokey Creek, which is also a tributary of the New River. Crabapple Creek and Bruce Creek are tributaries of Louse Creek, which is a tributary of the Clear Fork River. Both the New River and the Clear Fork River are tributaries of the Big South Fork of the Cumberland River. All six study streams are located within the borders of the North Cumberland Wildlife Management Area (NCWMA); specifically, the Campbell County streams are located within the Sundquist Unit (Fig. 1.2) and the Scott County streams (Fig. 1.3) are located within the Royal Blue Unit.

Crabapple Creek (Fig. 1.4) is an ecoregion reference stream, which is determined by TDEC to be the stream with the least amount of impact within a specific ecoregion. It is then used as a baseline comparison for gauging the impact to other streams within the same ecoregion (TDEC, 2006). There is no record of any mining activity in the vicinity of this stream, although there were recorded logging operations present in 1983.

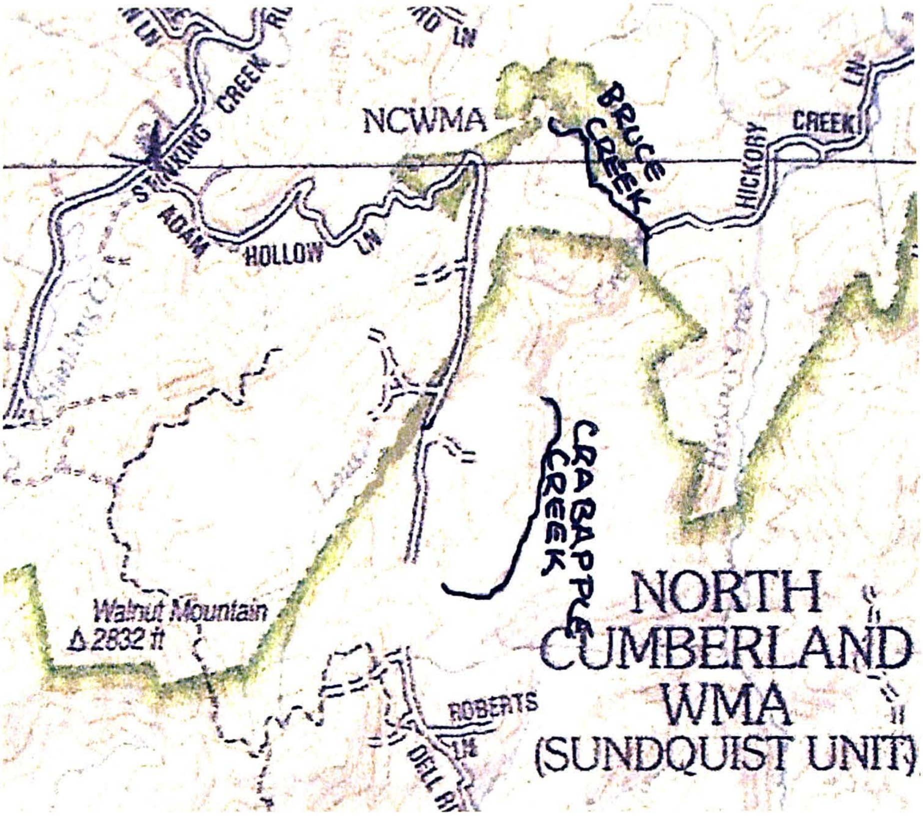


Figure 1.2. Study Streams in Campbell County TN. Includes the ecoregion reference stream, Crabapple Creek and the mined stream Bruce Creek. From DeLorme's *Tennessee Atlas and Gazetteer™*. Used with permission from the publisher. ©DeLorme, Yarmouth ME 04096.

Lowe Branch (Fig. 1.5), although not a TDEC ecoregion reference stream, has also been used as a reference stream in previous studies (Vaughan et al., 1982). The remaining streams have suffered varying levels of mining impacts over the last several decades (Table 3, Appendix D). Bruce Creek (Fig. 1.6) had less than 5% of its watershed impacted by surface mining circa 1965, prior to the passage of the Surface Mining Control and Reclamation Act (SMCRA) (Schiller, 1986; Whitley, 2009). Bowling Branch (Fig. 1.7), like Bruce Creek, suffered only small impacts from mining activity prior to 1977, but about

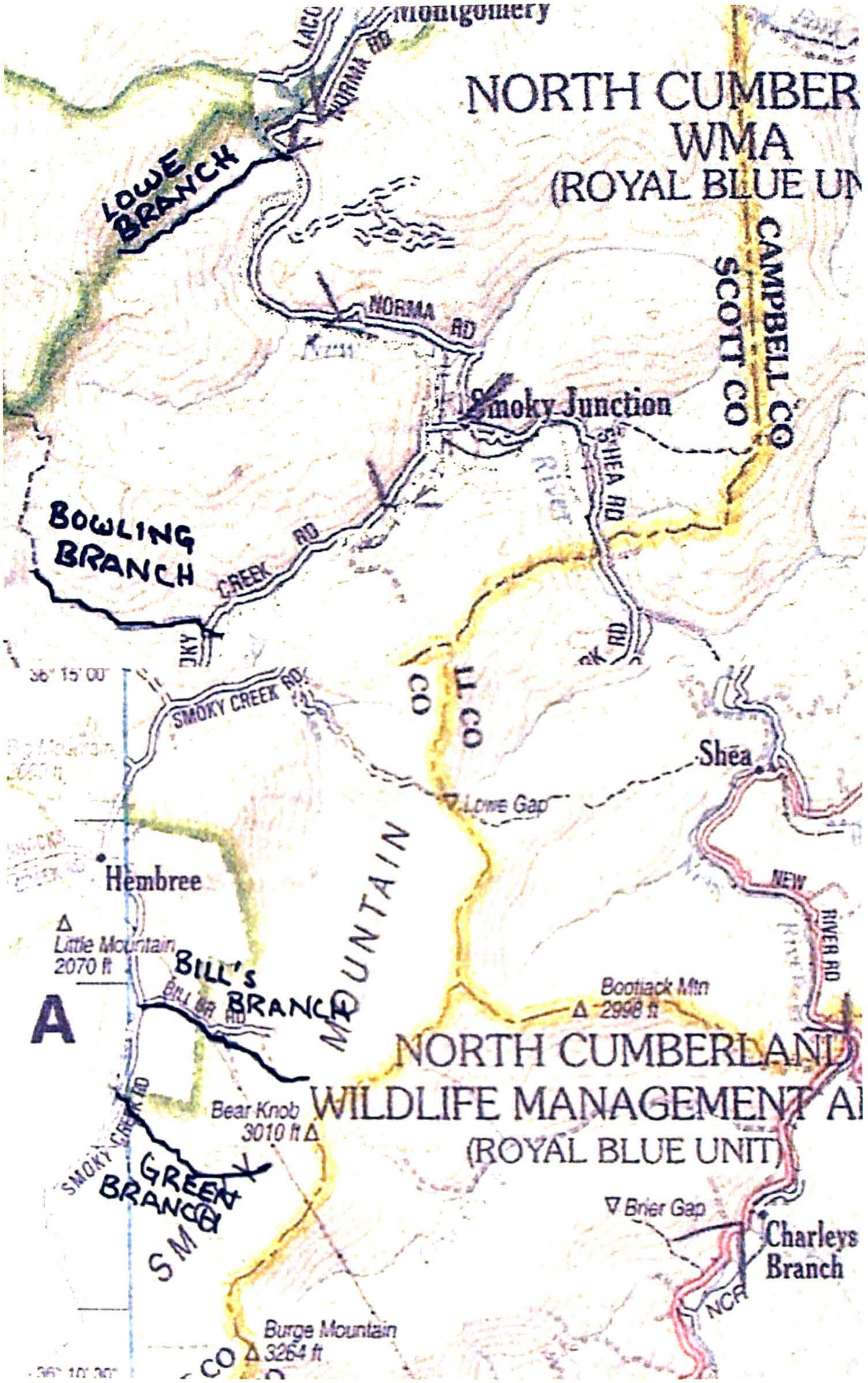


Figure 1.3. Study streams in Scott County, TN. Includes un-mined stream Lowe Branch, the mined streams Bowling Branch, Bill's Branch, and Green Branch. From DeLorme's *Tennessee Atlas and Gazetteer™*. Used with permission from the publisher. ©DeLorme, Yarmouth ME 04096.

3.2% of its surface area was disturbed by mountaintop mining in 1977-1978 (Dickens et al. 1989).

Bill's Branch (Fig. 1.8) had recorded mining activity during 1974-1975 that disturbed 9.8% of the watershed (Minear and Tschantz, 1976). Mining operations resumed in the Bill's Branch watershed in 2004; however, data detailing the percentage of disturbance in the Bill's Branch watershed in 2004 was not available. Green Branch is the most heavily impacted stream with mining activity during 1972-1975 that disturbed 24.1% of the watershed (Minear and Tschantz, 1976). Comparison of photos from 2008 and 2011 illustrates damage to the riparian vegetation downstream of the sampling sites on Green Branch (Fig. 1.9) caused by the recent restructuring of the roadway to accommodate heavy truck traffic associated with logging and mining. Mining operations resumed in the Green Branch watershed in 2007; however, like the Bill's Branch 2004 mining activity, data on how much of the Green Branch watershed was disturbed was not available.

The streams in this study have been affected by a variety of mining practices as technology and regulations evolved. Bill's and Green Branches have each been affected by more than one type of mining and reclamation, because they were mined repeatedly over time. See "Legislative Actions" below for details.

COAL MINING IN THE CUMBERLAND MOUNTAINS

Surface coal mining (contour mining) in the Cumberland Mountains of East Tennessee has been a local cultural and economic staple since the 1940's (Vaughan et al., 1982; Minear and Tschantz, 1976). This style of coal mining entails the removal of all vegetation, top soil and rock (overburden) to expose underlying coal seams.

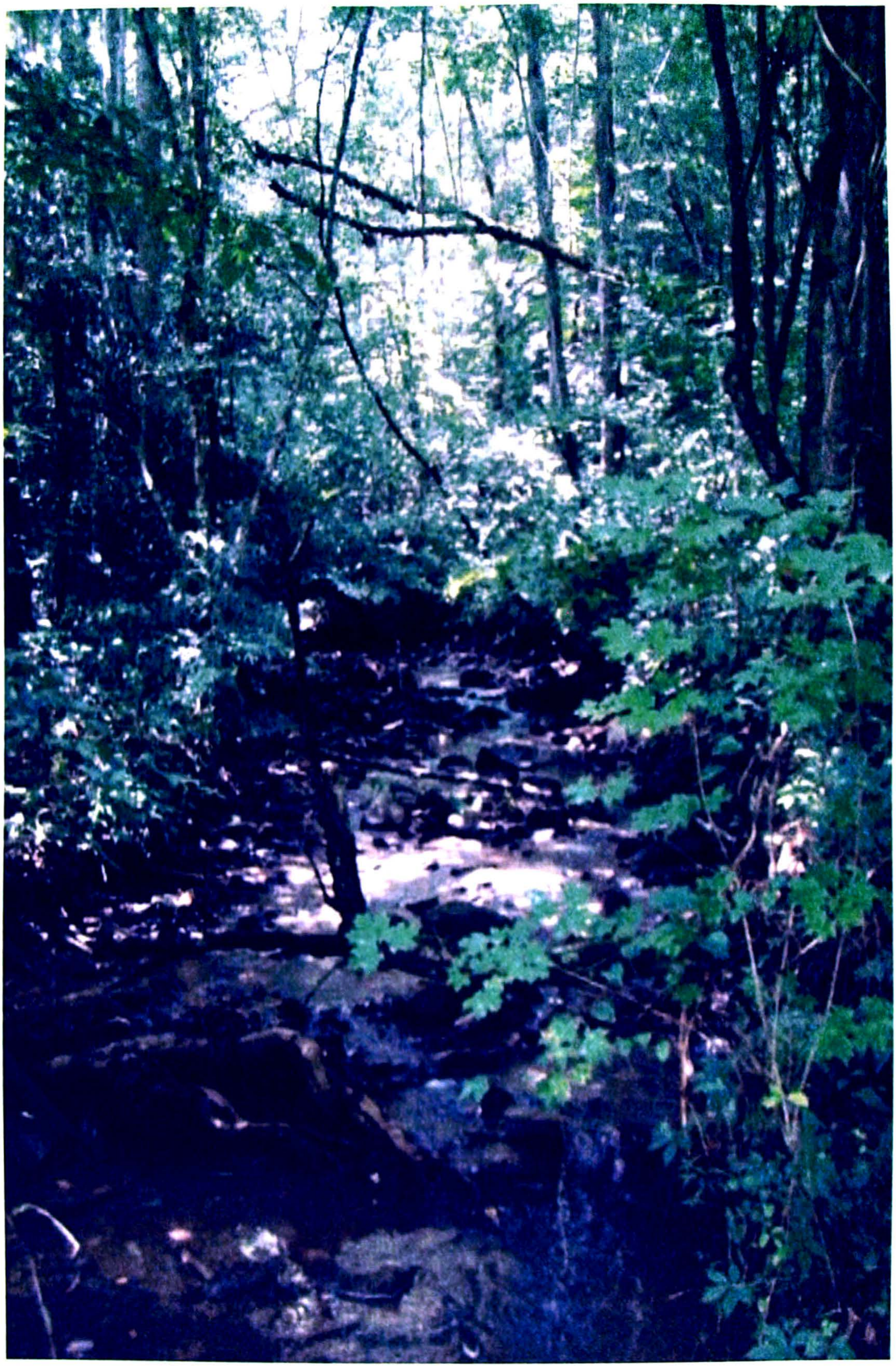


Figure 1.4. Crabapple Creek, Campbell County, TN

Figure 1.5. Bruce Creek, Campbell County, TN

Blasting dislodges massive amounts of spoil (soil, vegetation, and rock), resulting in increased sedimentation and damage to streams in the affected drainage basins. Once stripping was completed, only bare earth and rocks, high walls, benches and spoil banks remained in the areas mined prior to implementation of the SMCRA in 1977 (USGS, 1982) (Fig. 1.10). In some mining sites, additional coal was extracted by augering (Fig. 1.11). Augers are rotating bits that range in size up to seven feet in diameter and are used to bore into the mountain to bring out as much coal as possible (Caudill, 1971).

Figure 1.6. Lowe Branch, Scott County, TN

Post-SMCRA, many of the old mining practices such as pushing the overburden off the mining bench and down the mountain were outlawed and replaced by conservation oriented practices. An example of a typical post-SMCRA mining operation can be seen in Figure 1.12.

Figure 1.7. Bowling Branch, Scott County, TN

LEGISLATIVE ACTIONS

Prior to 1972, unregulated "cast overburden" was the general practice of mining operators (Figure 1.10). The cast overburden method entailed the disposal of spoil by simply pushing it over the side of the mountain (Dickens et al., 1989). Green Branch was affected by this method in earlier mining operations (Dickens et al., 1989). In 1972, Tennessee adopted the "swale backfill" method of reclamation (Dickens et al., 1989).

Figure 1.8. Bill's Branch, Scott County, TN

The swale backfill method modified the cast overburden method and created depressions along the mining bench that were designed to collect runoff and reduce flooding (Dickens et. al, 1989). The reclamation methodology changed again in 1974 as Tennessee adopted partial backfill procedures that were known as pasture and terraced backfill (Dickens et al., 1989). The mine operators separated acid-forming spoil and backfilled this spoil adjacent to the high wall (Dickens et al., 1989).

Figure 1.9. Green Branch, Scott County, TN. Photographs taken in 2008 (above) and 2011 (below).

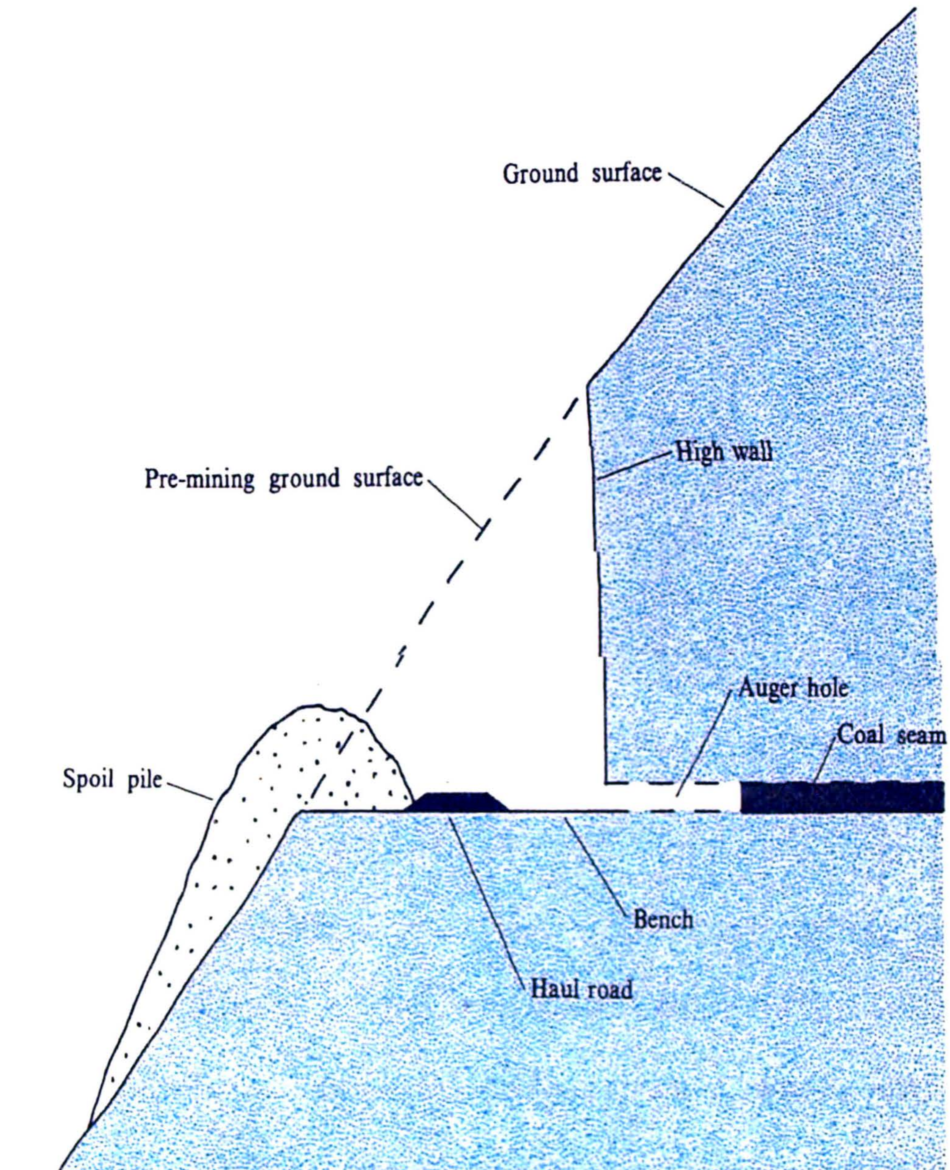


Figure 1.10. Diagram of a typical pre-SMCRA contour mining site. The illustration displays all alterations to the mountain: formation of the highwall, haul road and mining bench as well as the spoil pile that has been pushed over the side of the mountain (USGS, 1982).

Figure 1.11. Photograph of augering. Used to retrieve as much additional coal as possible from seam. Photo accessed 2 February 2014 (www.rosamine.org).

After 1975, mine operators were required to perform the terraced backfill reclamation process (Dickens et al., 1989). When SMCRA passed in 1977, specific reclamation efforts became a requirement for all types of mining, including mountain top removal (MTR) and surface contour mining (strip mining). The requirements are as follows: "the discharge of spoil below the mining cut is prohibited. Acid-forming and toxic spoil materials must be segregated, treated, and placed at the base of the mining highwall away from the reconstructed spoil slope (restoration of slope's approximate original contour or AOC)" (Dickens et al., 1989). Thus, recent mining operations in Bill's

Branch (2004) and Green Branch (2007) were subject to the reclamation regulations of SMCRA.

Figure 1.12. Photograph of a typical active post-SMCRA strip mine. Reclaimed strip mining sites restored to AOC and re-vegetated can be seen in the background. Photo accessed 2 February 2014 (www.rlch.org).

Some states in the Appalachian regions affected by surface mining began implementing legislation to require mining operators to apply for and obtain permits for potential mining sites in the early 1970's. The Tennessee Mineral Surface Mining Law of 1972 was one such law. It did not, however, consider coal to be a "mineral" (TDEC, 1972). Since coal was not legally considered a "mineral" under this law, the state did not require coal mining operators to obtain a permit, meaning that there was no governmental regulation or oversight of either their methods or the damage done to lands and streams adjacent to and/or downstream of the operations (TDEC, 1972). It was not until 1977 that Congress passed and implemented SMCRA. SMCRA falls under Section 30 of the US Code, Chapter 25. Subchapter 5 of this legislation addresses the control of environmental impacts of surface coal mining (U.S. Code, 2009). The Abandoned Mine Reclamation clause states that the Secretary of Agriculture is authorized to enter into agreements of not more than 10 years with land-owners to provide "land stabilizing, erosion and sediment control, and reclamation through conservation treatments" (U.S. Code, 2009). This clause also addresses the acquisition and reclamation of land adversely affected by past coal mining practices.

The federal reclamation requirements for mining companies state that plans must be made for control of surface water drainage and water accumulation. Backfilling, grading, soil stabilization, and re-vegetation would help to mitigate this run-off by restoring the mining site to its approximate pre-mining condition. The slope of the land and the vegetation provide natural barriers to excess run-off. The vegetation specifically acts as an anchor for the soil, keeping it from being washed down the mountain by heavy rains. It allows the water to flow down the mountain at a slower rate, and thus not overwhelm the streams with sediment. Descriptions of measures to be implemented during mining and reclamation assure the protection of: 1) quality of surface and ground water systems, both on- and off-site; 2) rights of present users to such waters; and 3) provisions for alternative sources of water where protection cannot be assured (U.S. Code, 2009).

There also exists a clause addressing "Areas Unsuitable for Mining" which declares that states may establish planning processes to declare land unsuitable for all or certain types of coal mining based on competent and scientifically sound data (U.S, Code, 2009). Tennessee has petitioned the federal Office of Surface Mining (OSM) to declare most of the New River watershed as areas unsuitable for mining, but this request is still pending as of this writing.

Ultimately, in the state of Tennessee, the issuance of all surface coal mining permits rests with the Office of Surface Mining. However, the application process for a surface coal mining permit involves several state and federal agencies. In 2009, a document called the National Memorandum of Understanding (MOU) was signed by the Department of the Interior, Department of the Army, and the Environmental Protection Agency (EPA) (TDEC, 2004). The purpose of this MOU was to "reduce the harmful environmental consequences of Appalachian surface coal mining operations, while ensuring that future mining remains consistent with federal laws" (TDEC, 2004). Within these guidelines, an agreement was reached between several local agencies. Included in this agreement were TDEC, the Nashville District of the U.S. Army Corps of Engineers (USACE), The Cookeville Field Office of the U.S. Fish and Wildlife Service (USFWS), The Knoxville Field Office of the Office of Surface Mining Reclamation and Enforcement (OSM), and Region 4 of the EPA. These agencies are linked by a mutual agreement named the Local Interagency Working Agreement (LIWA), whose purpose is to "improve agency communication and coordination during the coal mining permitting process in Tennessee under the respective state and federal permitting, enforcement, and compliance reviews required by the Clean Water Act (CWA), SMCRA, and the Endangered Species Act (ESA)" (TDEC Division of Water Pollution Control, 2004).

In addition, the State of Tennessee enacted the Responsible Miner's Act in 2009, which amended the original Tennessee Water Quality Control Act to include the protection of streams encountered during coal mining (TDEC, 2004). The Responsible Miner's Act states that: "(1) No permit shall be issued that would allow removal of coal from the earth from its original location by surface mining methods or surface access points to underground mining within one hundred feet (100) of the ordinary high water mark of any stream or allow overburden or waste materials from removal of coal from the earth by surface mining of coal to be disposed of within one hundred feet (100) of the ordinary high water mark of a stream...however, a permit may be issued or renewed for stream crossings...for operations to improve the quality of stream segments previously disturbed by mining and for activities related to and incidental to the removal of coal from its original location...", and "(2) Without limiting the applicability of this section, if the commissioner determines that surface coal mining at a particular site will violate water quality standards because acid mine drainage from the site will not be amenable to treatment with proven technology both during the permit period or subsequent to completion of mining activities, the permit shall be denied" (TDEC, 2004).

The permitting process is complex and lengthy, and involves water quality data collection, surface water chemical data, surface water biological data, surface water biological data for protection of threatened or endangered species (T/E), and groundwater data. All data gathered and submitted is presented as a Cumulative Hydrologic Impact Assessment (CHIA), which OSM will use as a determining factor in approval and/or denial of the surface coal mining permit (TDEC, 2010). Each agency involved in LIWA has its' own role to play in the permitting process. Detailing each step of the permitting process is outside the scope of this study; however, a brief overview of the documentation required and the agencies responsible for the certification of that documentation is necessary to have a basic understanding of the permitting process. The Clean Water Act (CWA) of 1972 contains specific sections that pertain to industry as it affects the nation's waterways or "waters of the United States". Coal mine operators must satisfy the requirements of CWA sections 401, 402 and 404. CWA section 401 refers directly to water quality standards set by the EPA and ensures that any impacts from the mining activity are minimized (EPA, 2010). TDEC is responsible for the approval and/or denial of the CWA 401 certification. TDEC also requires, as a part of the CWA 401 certification, that applicants have applied to the USACE for a CWA 404 permit, and that they submit an Aquatic Resource Alteration Permit (ARAP) to TDEC (TDEC, 2010). CWA section 402 addresses the National Pollutant Discharge Elimination System (NPDES), which regulates the point source discharge of pollutants into waters of the United States (EPA (a), 2014). TDEC has been named the regulatory authority for CWA 402 (TDEC, 2010). Since the regulatory authority rests with TDEC, the EPA retains authority for CWA 402 compliance (TDEC, 2010). CWA section 404 regulates the discharge of dredged and fill materials into waters of the United States, including wetland areas (EPA (b), 2014). Responsibility for compliance with CWA 404 is shared between EPA and USACE. EPA develops and interprets environmental criteria used in the evaluation of applications, while USACE is responsible for the day-to-day administration of individual permit applications and jurisdictional determination (EPA (b), 2014). Under the CWA, the EPA has veto authority for projects proposed under sections 402 and 404 (TDEC, 2010).

HYDROLOGIC EFFECTS OF SURFACE MINING ON STREAMS

Surface mine excavations remove the existing topsoil and forest vegetation and then fractures the underlying rock with explosives and removes it to expose the coal seam (Dickens et al., 1989). This material (overburden or mine spoil) is backfilled into the mine cut to restore the AOC during reclamation. Permeable geologic spoil acts as a water reservoir and picks up sediment and mineral constituents as it flows downhill (Dickens et al., 1989). The backfilled spoil, because of its fractured nature has tremendously increased the surface area exposed to weathering. Studies of the weathering of this spoil material indicate that "the weathering of spoil materials can dissolve an appreciable portion of the spoil mass at a rate orders of magnitude greater than normal soil weathering processes" (Dickens et al., 1989). The re-contouring of the mountainside mechanically alters the existing water flow-paths, both overland and within the backfilled mine spoils. Efforts to re-vegetate the area generally include the planting of non-native grasses, which do not control weathering and erosion of the soil as the original native forest vegetation did (Bernhardt and Palmer, 2011). Regardless of the regulatory changes, active mining operations still cause increased run-off and sedimentation resulting from the hydrological changes to the watershed (EPA (a), 2011).

In summary, where mined streams are compared to un-mined streams, the large reservoir of water held in the weathering mine fill, coupled with reduced interception and evapotranspiration of rainfall caused by forest clearing along with increased run off from the compacted backfill, leads to increased stream flows with higher TDS and sediment. All of these factors contribute sediment to the streams (Murphy et al., 2012; Bernhardt and Palmer, 2011; Lindberg et al., 2011; Dickens et al., 1989; Minear and Tschantz, 1976). Stream flows in mined watersheds are higher than those in un-mined watersheds and this increases the permanence of their flow during drought. In the New River Basin, streams impacted by mining will maintain measurable flow, even during periods of drought; however, undisturbed streams and reference streams routinely go dry during periods of little or no rainfall (Dickens et al., 1989). Whitley (2009) conducted a study in the New River Basin during a severe drought and the higher base flow in mined streams allowed sample collection in late spring, but the flow in un-mined reference streams was too low to sample. Only after large early summer rain events was it possible to collect samples from the un-mined streams.

The increased surface runoff in mined streams increases the flashiness. Even small amounts of rainfall may cause high flow in the stream and large amounts of rain cause local flooding. Studies have determined that run-off from mine sites can be much higher than in undisturbed areas; for example: five inches of rain in a disturbed area would have the same effect as would fifteen to twenty-five inches in an undisturbed area (EPA, 2012).

WATER QUALITY EFFECTS OF COAL MINING

Water released from storage in mine spoil enters the groundwater and which flows below ground to the stream channel. When this groundwater enters the stream as stream-flow, minerals such as iron and manganese are exposed to oxidation and precipitation (Dickens et al., 1989). In the Cumberland Mountains, coal contains pyrite (FeS_2). Pyrite produces sulfuric acid (H_2SO_4), which dissociates into hydrogen (H^+) and sulfate (SO_4^{2-}) ions (Lindberg et al., 2011). Because of the high concentrations of carbonates in the Cumberland Mountains, much of this acidity is neutralized. This results in alkaline mine drainage containing high concentrations of calcium, magnesium and bicarbonate, increasing the hardness of the water (Lindberg et al., 2011), and along with weathered spoil minerals results in higher levels of conductivity and total dissolved solids (TDS) compared to water in un-mined reference streams (Lindberg et al., 2011). Lindberg et al. (2011) concluded that stream water conductivity was positively associated with minerals derived from rock and coal weathering such as sulfates, calcium and magnesium (Murphy et al., 2012; Lindberg et al., 2011). Selenium and total dissolved nitrogen were also positively correlated to conductivity levels in the streams (Lindberg et al., 2011). Dickens et al. (1989) concluded that the amount of minerals stored in mine spoil may delay for a period of many years, the full extent of the water quality changes caused by surface coal mining. Recovery of water quality in mine-impacted streams depends on the amount of mineral constituents in the spoil and the amount of time it takes for the watershed to disperse the stored mineral constituents (Dickens et. al, 1989).

BIOLOGICAL EFFECTS OF COAL MINING

Headwater streams are the "birth place" of every river (Bernhardt and Palmer, 2011). These small interconnected water networks are essential to the health of larger streams and rivers in that they are the beginning of the food web that sustains larger waterways (Bernhardt and Palmer, 2011). When headwater streams are damaged by anthropogenic activities such as surface coal mining, the adverse effects are passed down the food webs to downstream segments of rivers (Fig. 1.13) (Bernhardt and Palmer, 2011). Changes in land-use, such as mining, increase stream-flow, sediment, and dissolved chemicals that flow to ecosystems downstream (Murphy et al., 2012; Bernhardt and Palmer, 2011; Lindberg et al., 2011; Dickens et al., 1989).

Alterations in stream hydrology and increased sediment, as explained in previous sections of this chapter, also degrade stream microhabitats (small, habitable locations within the larger stream habitat) essential to the various macroinvertebrate species in the streams. Some examples of these habitats include under rocks in riffles, the interstitial spaces between gravel and cobble, and accumulations of allochthonous carbon in the form of leaf packs and woody debris of various sizes. These habitats are degraded when excess sediment accumulates in the interstices of the substrate (embeddedness), covers the substrate surfaces (sedimentation), or excessive flow velocities scour and destabilize streambed substrates and flush allochthonous carbon from the stream reach. Macroinvertebrates will either drift to occupy a similar, less damaged habitat, or perish. Embeddedness and sediment deposition in pools reduces the "roughness" of the stream channel and increases flow velocity. Increased stream velocity may dislodge filter feeders and other macroinvertebrates (Tolbert, 1980; Talak, 1977). Embeddedness also reduces the permeability of the substrate which reduces the exchange of dissolved oxygen and nutrients between the water column and hyporheos, which in turn, interferes with important microbial and geochemical processes there.

Biogeochemical cycles, such as the carbon and the nitrogen cycles, are impaired in mined streams. Bacteria in the hyporheos (*Nitrobacter* spp.) help sequester toxic forms of nitrogenous waste (ammonia and/or ammonium) within the substrate. If the substrate has become embedded because of excess sedimentation, then this sequestration is impaired. Deposition of sediment on the surface of the substrate interferes with photosynthesis by diatoms and other algae, impairing autochthonous carbon formation. The effects of embeddedness and sedimentation include smothering algal growth, destruction of rooted plants, and interference with hatching and/or development of fish eggs (Talak, 1977).

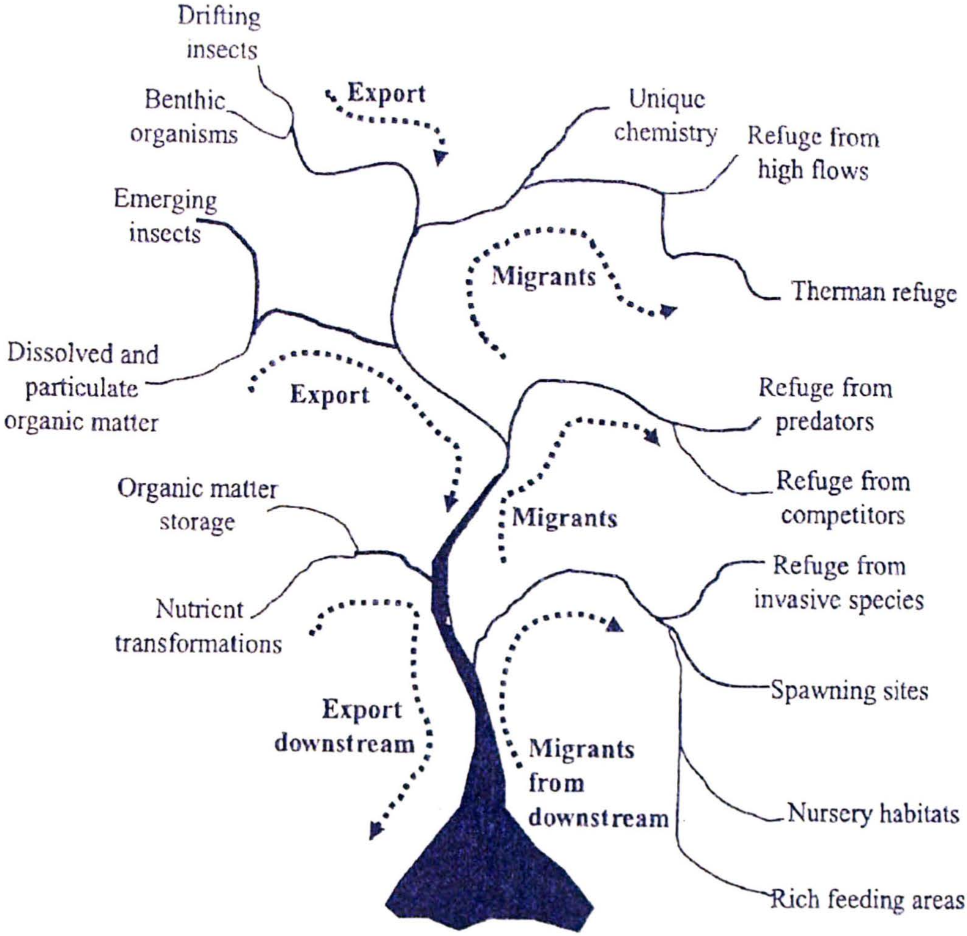


Figure 1.13. Illustration of how headwater streams contribute to the health of larger streams and rivers via food webs. Headwater streams are illustrated at the top of the figure, gradually moving to larger waterways downstream and finally into large rivers at the bottom of the figure (from Bernhardt and Palmer, 2011).

Elevated sulfate levels caused by weathering of the mine spoil stimulate microbial sulfate reduction in stream sediments. An increase in the streams' sulfate levels causes an increase in the production of sulfides (Bernhardt and Palmer, 2011). Phosphorus is a limiting nutrient for plants and is normally found bound to iron in streams. The sulfides are a phytotoxic agent and compete directly with phosphorus in binding to iron (Bernhardt and Palmer, 2011). If unbound phosphorus levels in the stream increase, often the result is eutrophication of the stream (Bernhardt and Palmer, 2011). Sulfides also inhibit nitrification, which can lead to a toxic environment for the macroinvertebrates. If nitrification is inhibited, physiological damage may occur in the macroinvertebrates. All macroinvertebrates produce nitrogenous waste as a by-product of metabolism. Thus, when their environment (the stream) contains excess ammonia/ammonium like compounds, their osmoregulatory capabilities are hampered. They are simply swapping one toxic nitrogenous molecule for another.

High conductivity is toxic to many macroinvertebrates by interfering with osmoregulation (Bernhardt and Palmer, 2011). Mayflies in particular are sensitive to conductivity levels; the increases in ions disrupt water balance and ion exchange, causing stress and/or death (Pond, 2012; Bernhardt and Palmer, 2011; Pond et al., 2008).

HISTORY OF BIOMONITORING

The fact that human activities in watersheds cause hydrological and associated chemical changes that ultimately manifest as changes to the structure and function of biological communities has led to the development of biological assessment protocols to assess adverse impacts to streams. Biological monitoring, or biomonitoring, is the use of organisms to gauge the health and/or impairment of habitats (Merritt et al., 2008). Scientists first began using aquatic macroinvertebrates to assess water quality standards in Germany in the early years of the 20th century. In 1909 Kolkwitz and Marsson developed the idea of saprobity to describe the degree of pollution (Merritt et al., 2008). The saprobity index was then used in rivers to determine the amount of pollution present as a result of sewage contamination (Merritt et al., 2008). Repeated observation of the responses of the macroinvertebrates to pollution led to the concept of "indicator organisms". In the saprobity indices, the "indicator organisms" were pollution tolerant organisms. The saprobity indices were gradually replaced by species diversity indices, first advocated by Wilhm and Doris (1968), which made use of information theory to assess water quality. Wilhm and Doris (1968) used comparisons of species diversity indices from both clean and polluted streams to gauge water quality. The perceived advantage of using a species diversity index was that it was an objective, numerical approach that was easily reported. In such indices, identification of organisms to genus is sufficient because the diversity value changes only slightly when organisms are identified to species level (Mackie, 2001). The indicator organisms used in contemporary biotic indices are generally the most intolerant of pollution and their presence indicates an absence of pollution. Hilsenhoff et al. (1977) developed the Hilsenhoff Biotic Index (HBI) which provided an estimate of the weighted average pollution tolerance of the macroinvertebrate community by providing an estimate of the severity of impairment to the benthic aquatic community. Contemporary bioassessment protocols in the U.S. have now been derived in most states and employ a multimetric approach that incorporates several metrics, including, but not limited to measures of community pollution tolerance such as the HBI and the North Carolina Biotic Index (NCBI) (Lenat, 1993). Other metrics that may be included in the multimetric index include measures of species diversity and/or species richness, as well as metrics of trophic organization, habitat specialization, and life history of stream communities (Karr, 1981; Plafkin et al., 1989; Barbour et al., 1999). Multimetric biotic indices (multi-metric indices) were developed in the 1960's and 70's, mainly in Europe. The Trent Index developed by Woodiwiss (1964) has been modified and adapted for use by several countries, to include the Tennessee Stream Pollution Board (Mackie, 2001). Karr developed the Index of Biotic Integrity using metrics describing the composition of fish communities (Karr, 1981). Karr's approach was extended by Plafkin (1989) and Barbour (1999) to use multiple metrics of fish, macroinvertebrates, and periphyton communities in multimetric indices to assess pollution impacts to streams (Barbour et al., 1999; Plafkin et al., 1989; and Karr, 1981).

BIOASSESMENT IN TENNESSEE: TDEC PROTOCOL – 2006

The Tennessee Department of Environmental Conservation's (TDEC) state protocols for water quality testing require the use of a semi-quantitative kicknet (SQKICK) for collecting macroinvertebrates in headwater streams. This methodology differs from the Surber sampler in that it employs a 0.1 m^2, 500 micron D-frame "kicknet". The collector positions the net in a chosen riffle while disturbing the substrate immediately in front of the net to an approximate depth of 10 cm and for a distance of 0.5 meter upstream of the net, using hands or a brush to scrape clinging organisms off rocks (TDEC, 2006).

One to four productive habitats must be selected for sampling using either the SQKICK or Surber sampler collecting methodology (TDEC, 2006). All macroinvertebrates should be identified and analyzed following the Tennessee Macroinvertebrate Index (Table 2, Appendix D). The index consists of six metrics. Scores for each metric are summed to obtain the multimetric bioassessment score. TDEC's protocols for abiotic variables call for the measurement of temperature, specific conductivity, pH, and dissolved oxygen.

HISTORY OF BIOMONITORING IN THE BIG SOUTH FORK WATERSHED

Bioassessment in this watershed began with a survey of the macroinvertebrate communities of 24 streams conducted in early June and/or early July of 1976 by Talak (1977) with the exception of Crabapple Creek, which was first studied in 1979 (Vaughan, 1982); and Bruce Creek which was first studied in 1984 (Schiller, 1986). Subsequent studies have revisited various combinations of the streams first studied by Talak (Whitley, 2009; Schiller, 1986; Vaughan et al., 1982; Williams 1981; and Tolbert 1978), but others have not been re-examined.

Minear and Tschantz (1976) initiated studies on the hydrology and water chemistry of streams in the New River and similar studies followed (Murphy et. al. 2012; and Dickens et al., 1989). Beginning in the 1940's to 1975, coal mining in the New River watershed (Anderson, Campbell, Morgan and Scott counties of Tennessee) had impacted approximately five percent of the total basin area (Minear and Tschantz, 1976). These studies have provided valuable contributions to the understanding of how mining affects physical and chemical changes in streams that subsequently cause changes in the biological communities revealed by biomonitoring studies. They also provided valuable, independently obtained measures of water chemistry that corroborate those collected in this and earlier biomonitoring studies.

Studies conducted by Vaughan et al. (1982) during the period of 1979-1981 encompassed five of the streams examined by Whitley (2009): Crabapple Creek, Lowe Branch, Bill's Branch, Green Branch, and Indian Fork; and four of the streams examined in this study: Crabapple Creek, Lowe Branch, Bill's Branch, and Green Branch. Macroinvertebrates were collected using Surber samplers with 15 thread/cm mesh. Vaughn et al. (1982) collected eight paired, randomly selected, 0.2m^2 Surber samples from each stream on a monthly basis (weather permitting). Samples were preserved in the field in 95% ethanol and transported to the laboratory for identification. Abiotic measurements were collected by standard methodologies in use during the late 1970's and 1980's. Biotic data were analyzed using the Shannon-Weaver Diversity Index to assess the structure of the macroinvertebrate communities (Vaughan et al., 1982). Schiller (1986) examined Bruce Creek, which had been mined approximately 15 years prior, and one of the same reference streams, Crabapple Creek, studied by Vaughan et al. (1982). Schiller (1986) collected macroinvertebrates, abiotic data, and analyzed diversity using the Shannon-Weaver Index as in Vaughan et al. (1982); however, he also analyzed stream particulate organic matter (POM), functional feeding group composition, and secondary productivity of the most abundant macroinvertebrate species.

Collecting methodologies and assessment methods have changed since Vaughan et al. (1982). These changes in methodology necessitated investigations to reconcile all methods so that data collected by all these studies throughout the years could be compared. Whitley (2009) conducted bioassessments on a subset of the streams studied by Vaughan et al. (1982) and those studied by Schiller (1986) using a macroinvertebrate data set for each stream consisting of four kicknet samples and a macroinvertebrate data set consisting of eight paired Surber samples. Both data sets were collected from the same stream reach at the same time, and analyzed using TDEC's protocol. The results obtained from these analyses were not significantly different and, thus, validated the analysis of data sets collected with Surber samplers from earlier studies with contemporary multimetric bioassessment protocols developed with kicknets. Whitley (2009) also determined that the collection of four paired Surber samples instead of eight, as was done in all previous studies, was sufficient to satisfy TDEC's "200 pick" macroinvertebrate requirement. However, this study was conducted during extreme drought conditions and difficult sampling conditions that caused concerns about generalizing the conclusions of her study. During Whitley's 2008 sampling season, low water conditions in Lowe Branch and Crabapple Creek prevented sample collection in June, when the other streams were sampled. Bill's Branch, Bowling Branch, Green Branch, and Bruce Creek were sampled mid-June, and Bowling Branch, Bruce Creek, Lowe Branch, and Crabapple Creek were sampled in late July when rains finally restored sufficient flow to Lowe and Crabapple Creek to allow sample collection. Only kicknet samples were collected from Bruce Creek and Bowling Branch on both sample occasions, but kicknet and Surber samples were collected from all other streams (Whitley, 2009).

OBJECTIVES OF STUDY

The objectives of this study are to: 1) Conduct macroinvertebrate bioassessment of six streams in the Cumberland Mountains of East Tennessee; 2) Compare bioassessments among studies to assess: (a) the recovery of stream macroinvertebrate communities from strip mining disturbance, and (b) the effectiveness of current mine reclamation requirements; 3) Assess the effects of drought conditions on bioassessments conducted in 2008; and 4) Assess the variability of bioassessments on the same stream in the same season among years.

CHAPTER II

MATERIALS AND METHODS

MACROINVERTEBRATE SAMPLE COLLECTION

Macroinvertebrates were collected using Surber samplers as described by Vaughan et al. (1982) and Schiller (1986), except only four paired Surber samples were collected from each stream, instead of eight paired Surber samples as collected in previous studies (Whitley, 2009; Schiller, 1986; Vaughan et al., 1982; Talak, 1977; Williams, 1981; and Tolbert, 1978). Whitley (2009) determined that four paired Surber samples were sufficient to provide the 200 macroinvertebrates required in the TDEC bioassessment protocol. A paired surber sample was collected from each of four riffle areas in sampling reaches of approximately 100 meters to obtain eight Surber samples per stream. Individual Surber samples were labeled as to site, date, and Surber pair/riffle, and then preserved in the field with 10% formalin and transferred to 70% isopropanol in the laboratory before picking out the macroinvertebrates.

PHYSICAL AND CHEMICAL DATA COLLECTION

Abiotic data were recorded using an YSI Environmental Monitoring System (650QS Display/Logger) to measure pH, dissolved oxygen (% and mg/L), specific conductance (µS/cm), temperature ($^{\circ}$C), and total dissolved solids. Alkalinity and total hardness were titrated in the field using a Hach kit (model number FF-1A). Habitat assessments were performed by Whitley (2009) in 2008 in accordance with TDEC protocol (Table 4, Appendix D). No obvious changes to the habitats of the sampled reaches of the study streams had occurred since 2008 so habitat assessments were not repeated.

PROCESSING OF MACROINVERTEBRATE SAMPLES AND DATA ANALYSIS

All macroinvertebrates in each of the four paired Surber samples were identified to the lowest practical taxon, primarily genus, using standard taxonomic keys (Epler, 2010; Merritt et al. 2008; Gelhhaus, 2008; and Wiggins, 1996). Enumerated taxa were entered into Microsoft Excel spreadsheets to calculate the metrics of the Tennessee Macroinvertebrate Index (TDEC, 2006). The index consists of the following seven metrics: taxa richness (number of different taxa within the sample), EPT richness (number of taxa belonging to orders Ephemeroptera, Plecoptera and Trichoptera within the sample), EPT percent (% or proportion of EPT individuals in the sample), %OC (proportion of oligochetes and chironomid individuals in the sample), %NUTOL (proportion of nutrient-tolerant organisms in the sample), %Clingers (percent of organisms that build fixed retreats or have adaptations to attach to surfaces in flowing water), and the NCBI or North Carolina Biotic Index (the weighted average pollution tolerance of the stream macroinvertebrate community based on empirically derived pollution tolerance values ranging from 0-10, with 10 being the most tolerant to pollution) (TDEC, 2006). Numeric ranges for each of these metrics are assigned a number ranging from 0-6, with 0 being the lowest possible score. These metric scores are then summed to obtain the multimetric bioassessment score.

It is important to note that earlier studies by Schiller (1986), Vaughan, et al. (1982), Williams (1981), Tolbert (1978), and Talak (1977) focused on the insects and smaller crustaceans (e.g., isopods and amphipods) and did not enumerate organisms such as decapods, oligochaetes and turbellarians, with the exception of hemipterans inhabiting the surface film which were excluded. Thus, these organisms were excluded from the bioassessments reported here. In addition, ceratopogonids and chironomids were enumerated only to family. These taxonomic discrepancies mean the taxa richness metric is somewhat less than would be obtained for these streams by contemporary assessments. The %OC metric is somewhat smaller than it would be in contemporary assessments because it does not include oligochaetes (TDEC, 2006).

All studies of these streams prior to Whitley collected eight paired Surber samples, for a total of 16 Surber samples (Schiller, 1986; Vaughan, et. al., 1982; Williams, 1981; Tolbert, 1978; and Talak, 1977). Whitley (2009) collected eight paired surbers as well, along with four kicknet samples from the same sample reaches as per the TDEC bioassessment protocol. She determined that the collection of four paired surbers per stream was sufficient to meet TDEC's "200 pick" standard. However, Whitley's (2009) bioassessments were based on averaged metric scores of each of the eight paired surbers per stream. In this study, the four pairs of Surber sample data were composited and metrics were calculated from the composited samples. This comports somewhat more closely to TDEC protocol, with the exception that TDEC bioassessments are based on a 200 pick of composited kicknet samples. In order to reconcile the bioassessments of this study with previous studies, new bioassessments compositing the four Surber samples of Whitley (2009) and Vaughan et al. (1982) were calculated. Comparisons of Bowling Branch and Bruce Creek were excluded from this study based on the fact that Whitley (2009) sampled these two streams with kicknets instead of surbers.

TDEC's categorical scoring criteria (non-impaired, slightly impaired, moderately impaired and severely impaired) are based upon quartiles of the multimetric scores obtained for reference streams for the ecoregion in which the study occurs. TDEC assesses stream health using the following categorical scoring criteria for Ecoregion 69d: non-impaired (≥32), slightly impaired (21-31), moderately impaired (10-20), and severely impaired (<10). The scoring criteria can be used in all streams that fit the sample criteria for that bioregion and have at least 80% of their upstream drainage in the same bioregion (TDEC, 2006).

Statistical analysis of biological and chemical data was performed in Jump 11.2 (SAS, 2014). Streams were classified according to mining history (mined vs. un-mined). ANCOVA's were performed to test for differences in biotic and abiotic attributes between mined vs. un-mined streams over time, with year as the covariate. Graphical analyses were performed using Microsoft Excel 2010.

CHAPTER III

RESULTS

RESULTS FROM PREVIOUS STUDIES IN THESE WATERSHEDS

Biological surveys of streams in this watershed began over 30 years ago, beginning with a study conducted by Talak (1977). Subsequent studies on various combinations of the streams studied by Talak and two additional streams were conducted by Whitley (2009), Schiller (1986), Vaughan et al. (1982), Williams (1981), and Tolbert (1978). This study, conducted in 2011, furthers these efforts; therefore, results from this study will be presented together with those from earlier studies in order to illustrate temporal trends.

ABIOTIC DATA

pH IN MINED VS. UN-MINED STREAMS 1979, 1980, 1981, 2008 and 2011

The pH of streams measured in this and earlier studies (Fig. 3.1; Appendix D, Table 5) ranged from slightly acidic (6.4) to slightly basic (8.1). The pH values in this study were similar to those obtained in the earlier biomonitoring studies (Whitley, 2009; Schiller, 1986; Vaughan et al., 1982; and Tolbert, 1978). All values were recorded in late spring and are within the seasonal variation in pH for individual streams. These pH values are consistent with those reported for these streams by other studies (Dickens et al., 1989; Bradfield, 1986; and Minear and Tschantz, 1976).

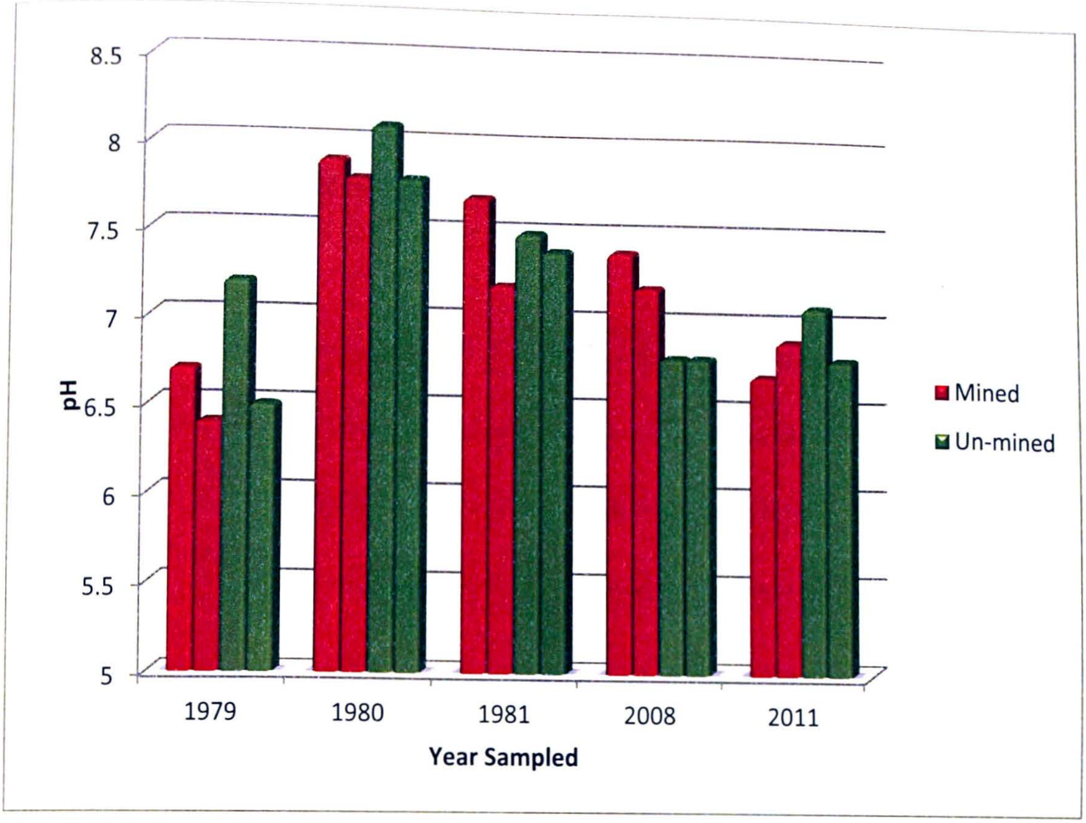


Figure 3.1. pH levels in mined vs. un-mined streams from 1979, 1980, 1981, 2008 and 2011.

<u>CONDUCTIVITY IN MINED VS. UN-MINED STREAMS 2008 and 2011</u>

Conductivity (specific conductance) is a measurement of water's ability to pass an electrical current. Since ions can conduct electricity, specific conductance is an indirect measurement of the concentration of ions in stream water (Dickens et al., 1989 and Minear and Tschantz, 1976). Conductivity of the un-mined reference streams was low, which is to be expected in streams with no or little anthropological disturbance. Conductivity was significantly different (p=0.0234) between mined and un-mined streams, with the mined streams displaying higher conductivity values. Conductivity values in all streams decreased between 2008 and 2011, most likely because the abnormally low flows during the drought conditions of the 2008 sampling increased the portion of stream flow consisting of groundwater that contains more weathered ions (Fig. 3.2; Appendix D, Table 6) (Murphy et al., 2012).

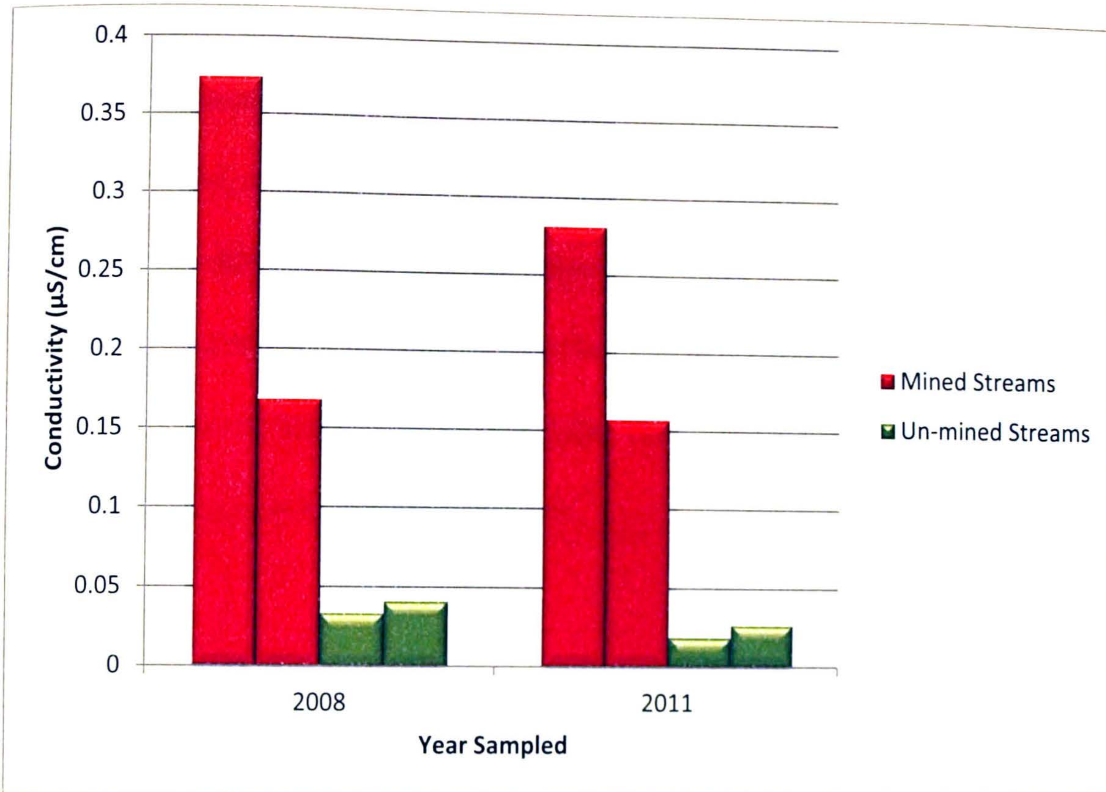


Figure 3.2. Conductivity in mined vs. un-mined streams from 2008 and 2011.

BIOTIC DATA

One objective of this study was to evaluate temporal trends over the time period encompassed by the various studies of these streams from 1979 to the present. Earlier studies provide consistent biotic data from 1979, 1980, 1981, 2008 and 2011 for four streams; Green Branch and Bill's Branch (mined streams); and Crabapple Creek and Lowe Branch (un-mined streams). Other streams in this study were not sampled consistently in all these years, or the data from earlier studies was not available, or the collection methodology was not comparable. An additional objective was to compare bioassessment results from 2008, a severe drought year, to those from other years.

TAXA RICHNESS IN MINED VS. UN-MINED STREAMS 1979, 1980, 1981, 2008 and 2011

Taxa Richness was generally greater in the two un-mined streams, Lowe and Crabapple creeks, compared to two of the mined streams, Green and Bill's branches, for each of the five different years (Fig. 3.3; Appendix D, Table 7). However, taxa richness trended higher until 2008 and then declined in three of these four streams: Green Branch, Bill's Branch, and Lowe Branch in 2008. Taxa richness decreased from 2008 to 2011, with the exception of Crabapple Creek.

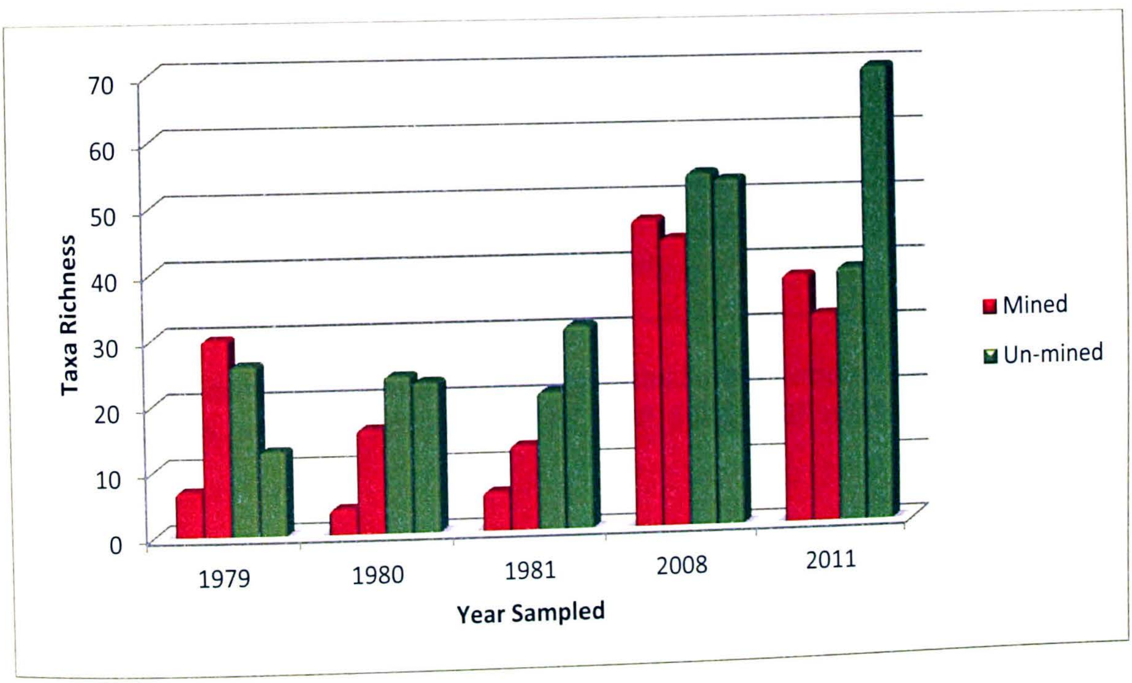


Figure 3.3. Taxa richness scores of 4 pairs of composited Surber samples collected from mined vs. un-mined streams in 1979, 1980, 1981, 2008 and 2011.

EPT RICHNESS IN MINED VS. UN-MINED STREAMS 1979, 1980, 1981, 2008 and 2011

EPT richness, like taxa richness, tended to increase in these four streams from 1980 through the most recent study (Fig. 3.4; Appendix D, Table 8).

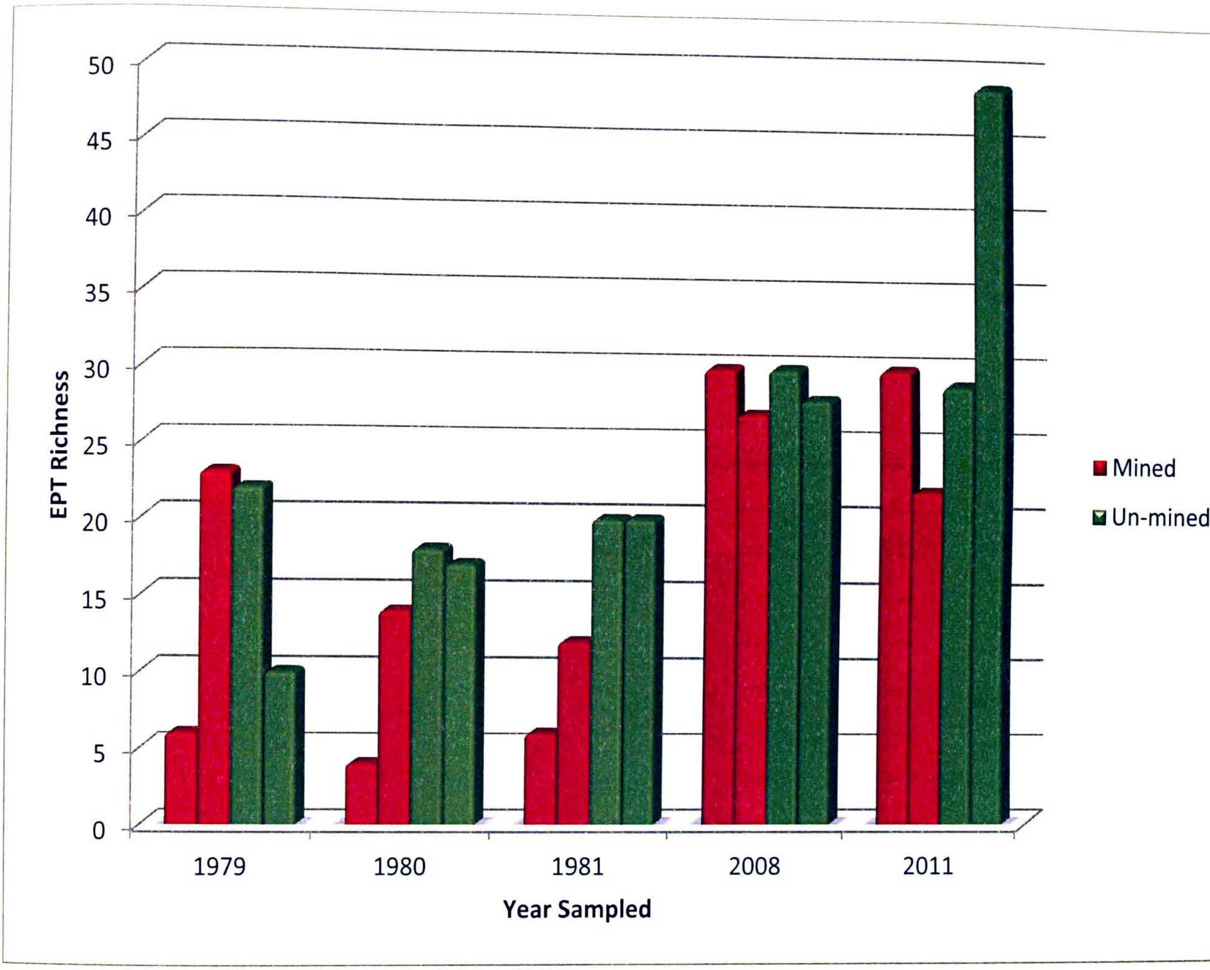


Figure 3.4. EPT richness of four pairs of composited Surber samples collected from mined vs. un-mined streams in 1979, 1980, 1981, 2008 and 2011.

MULTIMETRIC SCORES IN MINED VS. UN-MINED STREAMS 1979, 1980, 1981, 2008 and 2011

Multimetric scores displayed the same approximate pattern seen in diversity metrics of a consistent increase over time. The exceptions are Lowe Branch and Crabapple Creek, which declined in 2008 compared to 1981, but the multimetric scores indicate continuing recovery of the mined streams from 1980 to present (Fig. 3.5; Appendix D, Table 9). All multimetric scores fell within the unimpaired bioassessment classification with the exception of Green Branch in 1979 and 1980 and Lowe Branch in 2008. Bill's Branch was classified as moderately impaired in 1979, and slightly impaired in 1980. Lowe Branch was classified as slightly impaired in 2008. Multimetric scores in both Crabapple Creek and Lowe Branch display some variation among the sample years, but variations are likely due to weather and other differences. The dip in multimetric scores in 2008 may reflect the greater impact of drought on flows in un-mined streams.

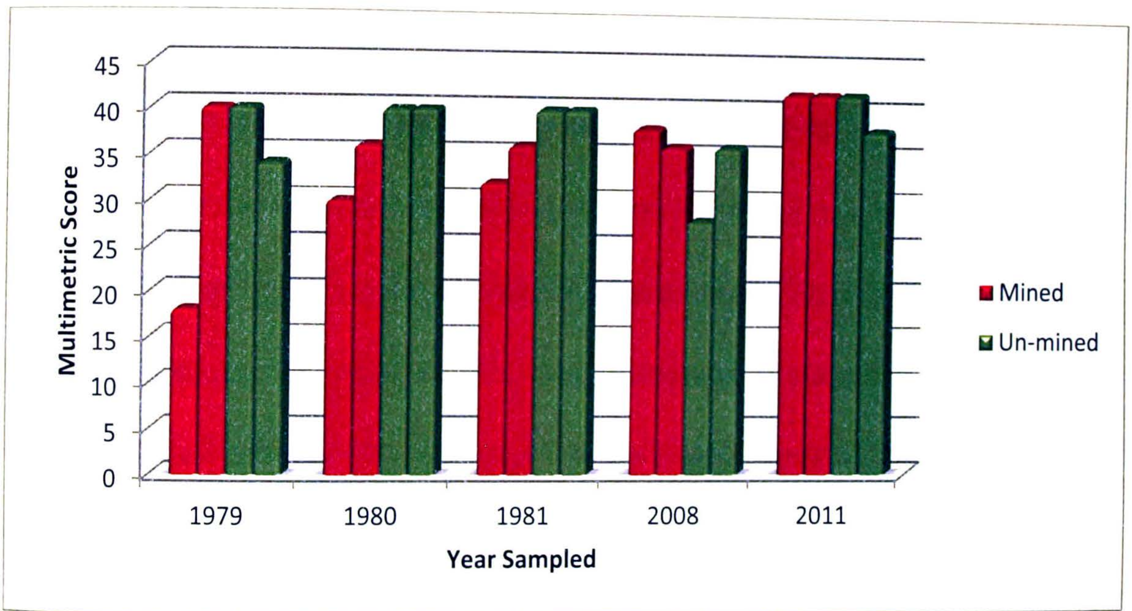


Figure 3.5. Multimetric scores of four pairs of composited Surber samples collected from mined vs. un-mined streams in 1979, 1980, 1981, 2008 and 2011.

STATISTICAL ANALYSIS OF MULTIMETRIC SCORES IN MINED VS. UN-MINED STREAMS

While a repeated measures analysis would be more appropriate for this data, the sample size in this study was too small to be statistically meaningful. Instead, an

ANCOVA was used to analyze each set of metric data as described in the previous chapter (Appendix C). Multimetric scores did not differ significantly among years or between mined and un-mined streams.

MACROINVERTEBRATE ABUNDANCE IN MINED VS. UN-MINED STREAMS IN 1979, 1980, 1981, 2008, 2011

The abundance of macroinvertebrates collected in four pairs of composited Surber samples (Fig. 3.6; Appendix D, Table 10) was significantly less in mined compared to un-mined streams (p=0.0011) when tested in an ANCOVA using sampled year as the covariate. There is also a significant difference in the Macroinvertebrate Abundance among Year Sampled analysis (p=0.0009) (See Appendix C for full analysis). While the lower abundance of macroinvertebrates in the mined streams may not affect any of the metric calculations, it is, in itself, an objective indication of a significant difference between mined and un-mined streams. Thus, abundance of macroinvertebrates contradicts the multimetric bioassessment results finding almost all the streams were not impaired.

In the Mining Status Leverage Plot (Fig. 3.7) no significant difference was found (p=0.0902) between mined and un-mined streams, although the LS Means Plot (Appendix C) does show the expected lower macroinvertebrate abundance in mined than in un-mined streams. The leverage plot for the Year Sampled reflects a steady increase in macroinvertebrate abundance from 1979 to 2008 and decline in 2011 (See Appendix C for full analysis).

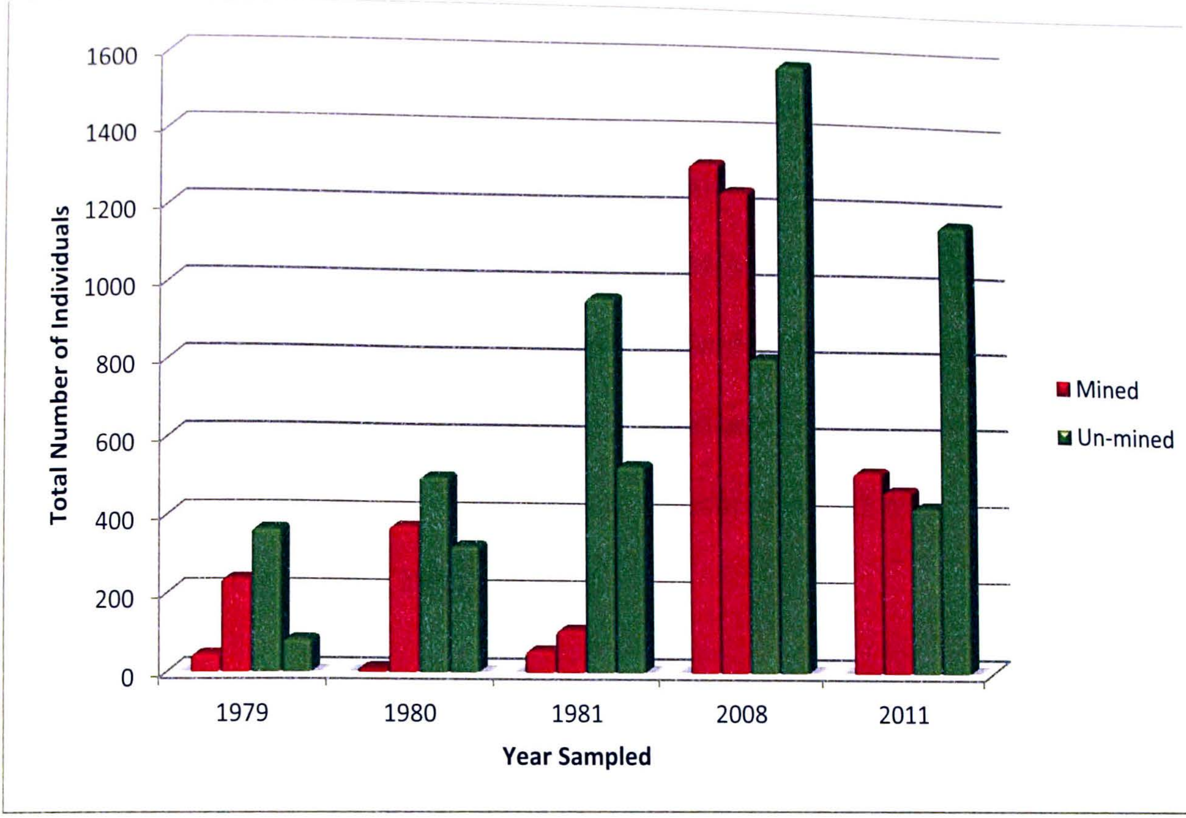


Figure 3.6. Total number of macroinvertebrates collected in four pairs of composited Surber samples collected from mined vs. un-mined streams in 1979, 1980, 1981, 2008, 2011.

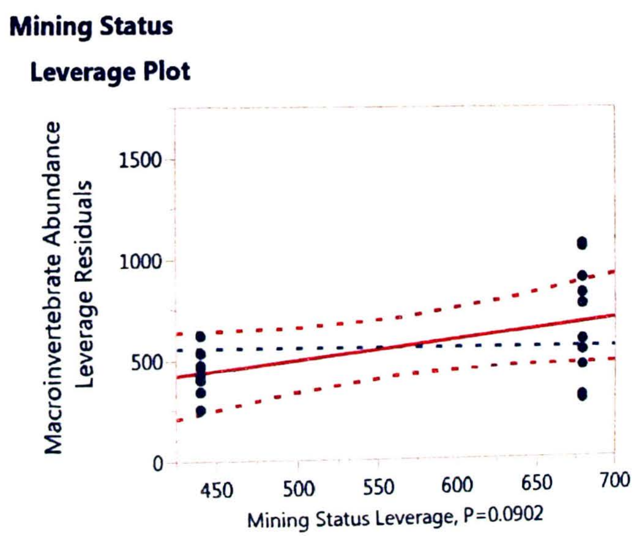


Figure 3.7. ANCOVA: Mining status leverage plot (p=0.0902). See Appendix C for full analysis.

The spike in abundance in 2008, again highlighting the effects of adverse weather conditions, and then in 2011, the abundance seems to pick up and follow the steady increase seen from 1979 – 1981. The 2011 abundance was, of course, much lower than in 2008. A Tukey HSD (Fig. 3.8) was included in the ANCOVA to pinpoint the years that were significantly different. It shows macroinvertebrate abundance in 2008 was not significantly different from 2011, but was significantly different from 1981, 1980, and 1979. Additionally, macroinvertebrate abundance in 2011 did not differ significantly from that in 1981, 1980, and 1979.

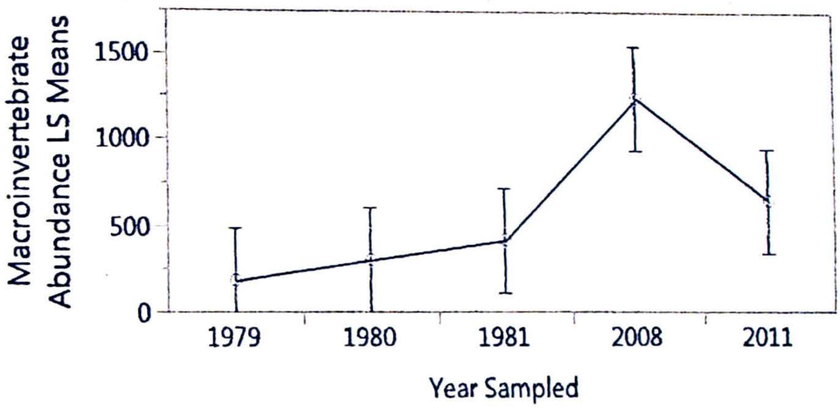
COMPARISON OF BIOASSESSMENT OF MEAN METRIC SCORES (WHITLEY, 2009) TO BIOASSESSMENT OF FOUR COMPOSITED PAIRS OF SURBER SAMPLES (PRESENT STUDY)

I repeated the bioassessment of the streams Whitley analyzed in 2008, by compositing four of the eight pairs of Surber samples she used in her bioassessments (Table 11, Appendix D). Whitley's (2009) bioassessments were based on the average metric scores of each of the eight pairs of Surber samples she collected from each stream. Whitley (2009) sampled Bruce Creek and Bowling Branch in June and again in July to serve as controls when she was unable to sample the un-mined streams, Lowe Branch and Crabapple, in June; however, it is important to note that all samples taken from Bruce Creek and Bowling Branch in 2008 were collected using the TDEC SQKICK methodology instead of Surber samplers; thus, bioassessments of these streams reported for 2008 were obtained through analysis not directly comparable to these results for these streams in 2011, but are reported here anyway because this is the only estimate of their condition available for 2008 (Appendix D, Table 15).

Response Macroinvertebrate Abundance

Year Sampled

LS Means Plot



Response Macroinvertebrate Abundance

Year Sampled

LSMeans Differences Tukey HSD

Level			Least Sq Mean
2008	A		1245.2500
2011	A	B	650.0000
1981		B	418.0000
1980		B	302.5000
1979		B	183.0000

Levels not connected by same letter are significantly different.

Figure 3.8. ANCOVA results: LS Means Plot of Macroinvertebrate Abundance vs. Year Sampled and LS Means Difference Tukey HSD.

There was little variation in multimetric scores of the two streams between the June & July sample dates and both streams were classified as slightly impaired on both dates (Whitley, 2009). Thus, it appears valid to compare the results for Lowe Branch and Crabapple Creek, which were only sampled in July, to the results from the other streams sampled only in June.

ANALYSIS OF CRABAPPLE CREEK AS A REFERENCE STREAM 1979, 1980, 1981, 2008 and 2011

Crabapple Creek is the least disturbed of any stream in this study. As such, it provides an indication of the variation in bioassessment outcomes that can be expected in the absence of disturbance. The following results provide an in-depth examination of how individual metric scores and the multimetric bioassessment scores may vary over a time scale of decades in one of the least disturbed streams in the region. Reference streams are a critical piece of bioassessment protocols. Their aquatic macroinvertebrate communities serve as the standard of comparison for all other streams in the region. This comparison allows us to gauge the severity of impact to a study stream. If a reference stream is unknowingly impaired by anthropogenic impacts, it compromises all bioassessments in that ecoregion because impaired streams are assessed as being more similar to reference condition streams.

TAXA RICHNESS IN CRABAPPLE CREEK 1979, 1980, 1981, 2008 and 2011

Taxa richness in Crabapple Creek (Fig. 3.9; Appendix D, Table 12) progressively increased beginning with Vaughan's (1979) study, and continued through the present study.

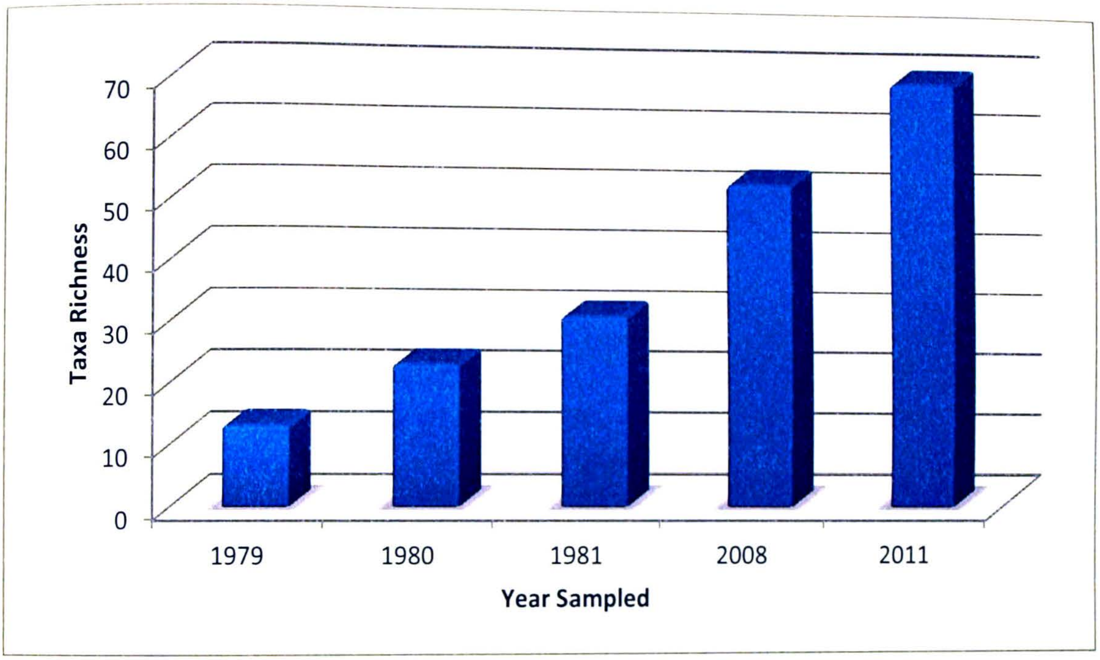


Figure 3.9. Taxa richness of four pairs of composited Surber samples collected from Crabapple Creek in 1979, 1980, 1981, 2008 and 2011. Crabapple is an un-mined, "pristine" ecoregion (69D04) reference stream.

EPT RICHNESS IN CRABAPPLE CREEK 1979, 1980, 1981, 2008 and 2011

EPT richness in Crabapple Creek (Fig. 3.10; Appendix D, Table 13) follows the same trend of gradual increase as did taxa richness above.

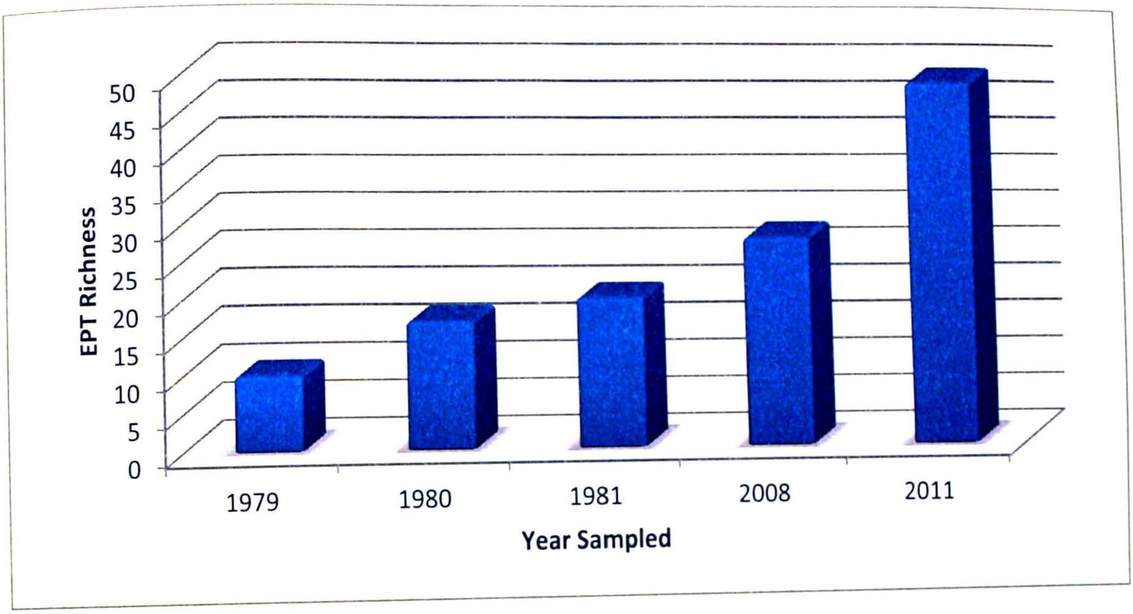


Figure 3.10. EPT richness in Crabapple Creek from 1979, 1980, 1981, 2008 and 2011. Crabapple Creek is an un-mined, "pristine" ecoregion (69D04) reference stream.

MULTIMETRIC SCORES IN CRABAPPLE CREEK 1979, 1980, 1981, 2008 and 2011

Multimetric scores (Fig. 3.11; Appendix D, Table 14) for Crabapple Creek ranged from a high of 40, to a low of 32, but all scores for all years classify Crabapple Creek as not impaired based on TDEC's non-impaired category (≥32).

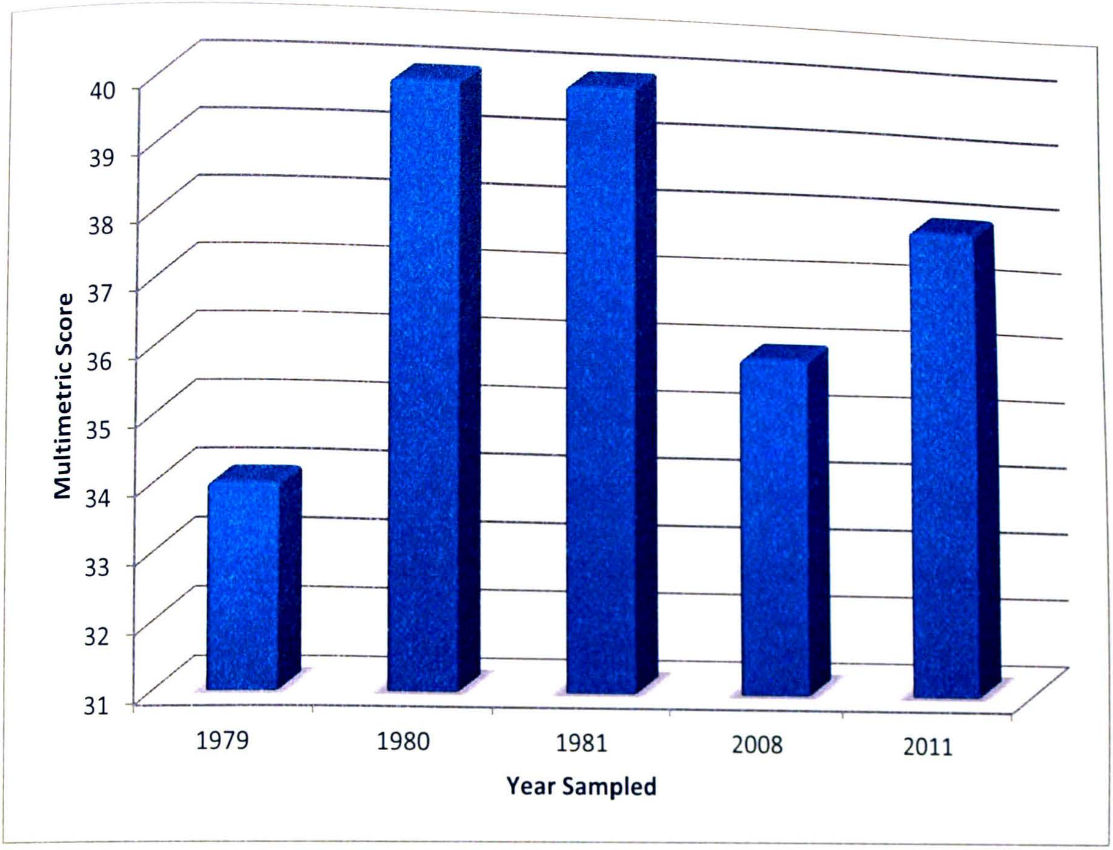


Figure 3.11. Multimetric scores for Crabapple Creek from 1979, 1980, 1981, 2008 and 2011. Crabapple is an un-mined, “pristine” ecoregion (69D04) reference stream.

CHAPTER IV

DISCUSSION

ABIOTIC VARIABLES

COMPARISON OF pH VALUES 1979, 1980, 1981, 2008 and 2011

Any type of coal mining method can produce acidic run-off due to chemicals released from the coal (i.e., sulfides), which can then make their way into the surrounding streams, reducing stream pH (Pond, 2008). In the Cumberland Mountains, however, surface coal mining normally does not produce acidic run-off unless sulfur-bearing rocks are abundant in the mined area of the watershed. This is because surface coal mining in the study area increases alkalinity in the streams by disturbing calcium carbonate-bearing rocks. Weathering of these rocks releases calcium carbonate into the streams which increases the buffering capabilities of the streams, preventing shifts in pH (Dickens et al., 1989; Minear and Tschantz, 1976). Murphy et al. (2012) suggests that some SMCRA reclamation requirements (returning to AOC) are not completely effective in remediating the abnormalities in abiotic factors, such as pH and conductivity, since these variables may remain elevated years and decades after mining operations have stopped (Murphy et al., 2012). Although pH increased in all streams from 1979 – 1980, there is no obvious cause to which this can be attributed. Given that the un-mined streams show a consistent increase as well, the increased pH could have been the result of weather conditions or equipment differences. Green Branch, Bill's Branch, Bruce

Creek and Bowling Branch have all experienced impacts from coal mining operations, most prior to the implementation of SMCRA in 1977; when mine operators were not required to implement any type of reclamation efforts. Dickens et al. (1989) discusses the three most common pre-SMCRA reclamation efforts, cast overburden, swale backfill and terraced backfill. These reclamation efforts and their chronology in respect to the passage of SMCRA in 1977 are discussed in detail in Chapter I. Mining activity resumed in the Green Branch and Bill's Branch watersheds in 2004 and 2007, respectively, and both were reclaimed, as required by SMCRA regulations. There was no obvious difference in stream pH between pre-SMCRA and post-SMCRA mining.

COMPARISON OF CONDUCTIVITY VALUES 1979, 1980, 1981, 2008 and 2011

A direct comparison of conductivity values over the entire 35 year history of monitoring is not possible due to changes in collection methodologies and technology. In earlier studies (Schiller, 1986; Vaughan et al., 1982; Williams, 1978; Talak, 1977), specific conductance was not directly measured. Instead, total dissolved solids (TDS) and turbidity of the water were considered proxies for conductivity. Concentrations of specific ions such as sulfates, calcium, magnesium, iron and manganese were measured in lieu of specific conductance. Murphy, Hornberger and Little (2012) found that "SC (specific conductance) and SO_4 (sulfate) have been documented as indicators of coal mining pollution in otherwise pH-neutral waters...in other words, in pH-neutral waters, sulfate concentrations explain at least 98% of the variation in SC" (Murphy et al., 2012).

Increases in iron, sulfates, copper, arsenic, selenium, cadmium and other ions have been documented in watersheds with active and abandoned mining operations (USGS, 2013; Murphy et al., 2012; Minear and Tschantz, 1976). All of the previously listed elements dissolve into water as ions, and as such, contribute to increasing conductivity (Minear and Tschantz, 1976). Mined streams in the study area have historically displayed levels of suspended solids in excess of 1,000 mg/L, occasionally exceeding 10,000 mg/L, while undisturbed watersheds were consistently below 25 mg/L (Talak, 1977). It is important to note that Talak (1977) completed his study before passage of SMCRA in 1977 and very little, if any, reclamation was applied so his study streams were subject to the unmitigated effects of mining.

Concentrations of metals and/or suspended solids were not analyzed by Whitley (2009) or this study. Instead, the measurements taken by Whitley (2009) and this study were conductivity measurements. However, in future studies, comparisons of TDS in mined vs. un-mined streams from all studies would be helpful in creating a more complete picture of the hydrological changes mined streams are experiencing, and could potentially explain some of the macroinvertebrate community changes within the streams.

Increases in conductivity of headwater streams can be due to run-off from mining, logging, and agricultural activities (Minear and Tschantz, 1976). Murphy et al. (2012) found that reclaimed mined lands continue to leave a "legacy" effect on hydrology and conductivity for years. When the mining companies reclaim the mined area by using mine spoil to backfill and return the land to the AOC, the soil is compacted by the heavy machinery. This retards the infiltration of water during heavy rain events and increases surface runoff and erosion. Water does gradually infiltrate the compacted mine fill and dissolves ions from the weathering of the spoil as it percolates through the now artificial mountainside (Murphy et al., 2012). These ions are transported into the groundwater downslope of the mine fill as they make their way into the stream (Murphy et al., 2012; Dickens et al., 1989). Thus, the groundwater in the mine fill and all the permeable substrate of the watershed downslope from the mine fill becomes a large reservoir of elevated dissolved solids that can be expected to increase conductivity of stream water for many years and possibly many decades. Murphy et al. (2012) observed improvements in conductivity and sulfate levels over the last 3 decades in the Indian Fork watershed and hypothesized that the mine spoil contains a finite amount of readily weatherable materials that are becoming depleted in the mine spoil and this may cause levels of conductivity and sulfates to continue to decline over time.

BIOTIC VARIABLES

TAXA RICHNESS SCORES 1979, 1980, 1981, 2008 and 2011

Bill's Branch and Green Branch were both mined circa 1972-1975; however, taxa richness was lower in Green Branch compared to Bill's Branch because a much larger fraction of the watershed was mined in Green Branch (Vaughan et al., 1982). Bill's Branch watershed encompasses 174 hectares and 9.8% of its watershed was disturbed by mining, whereas, Green Branch watershed encompasses 357 hectares and 24.1% of its watershed was disturbed by mining (Minear and Tschantz, 1976). This comparison indicates the adverse effects to the macroinvertebrate community are a function of the percentage of watershed disturbed by mining.

Taxa richness in Bill's Branch declined in each year it was sampled by Vaughan (1982), but was dramatically increased when sampled in 2008 (Whitley, 2009). The progressive declines in taxa richness observed by Vaughan (1982) may reflect the time delay between mine disturbance of the watershed and the manifestation of the effects in the stream. Effects such as increased siltation, conductivity, and flashiness could all have been increasing during the time Vaughan (1982) studied Bill's Branch. A great deal of recovery apparently occurred during the long time interval between then and Whitley's (2009) study as judged by the high taxa richness scores.

Whitley (2009) collected during a drought year, 2008, and the low stream flows prevented sampling of the un-mined study streams at the same time as the mined streams. The flow in the mined streams was not as affected because of the hydrological changes to these streams caused by mining. A subsequent sampling of the un-mined streams was undertaken immediately following the next significant rain events in July. Lowe Branch was sampled just as a spate was ending and high flows were present, but did not seem to interfere with the efficiency of macroinvertebrate collection given the numbers and diversity of macroinvertebrates collected. However, these adverse weather conditions in 2008 may have, paradoxically, allowed for an unusual abundance of macroinvertebrates to be collected. There are several possible reasons for the increase in taxa richness in 2008. First, in 2008, the samples were collected much later in the year (June and July) than those in 2011 (April). Perhaps a more diverse macroinvertebrate assemblage is typical of summer compared to spring. Many species may begin their development during summer resulting in larger numbers of earlier instars that are more difficult to identify and may have been misidentified resulting in an inflated estimate of taxa richness. It is also possible this much variation in taxa richness between years occurs naturally in these streams and has not been documented before because of the infrequent biomonitoring of the same streams. This possibility could be verified by re-examining the earlier collections of Schiller (1986) and Vaughan (1982) during the same months to establish if there is a consistent pattern. Another possibility is that the macroinvertebrates were more concentrated in the areas that were suitable for sampling because of the low stream flows.

The decrease in taxa richness in all but Crabapple Creek between 2008 and 2011 could represent a return to more typical taxa richness levels because macroinvertebrates were less concentrated in a reduced available habitat. However, extensive logging in these watersheds occurred in the interval between these two studies and may also explain much of the reduction of taxa richness observed in 2011. While the extent of logging in the mined watersheds and in Lowe Branch watershed was not determined at the time of macroinvertebrate collection in 2011, it is known with certainty that logging had not yet reached Crabapple Creek watershed in 2011, the only stream of the four with even higher taxa richness than in 2008.

EPT RICHNESS SCORES 1979, 1980, 1981, 2008 and 2011

EPT richness increased progressively most years sampled with the exception of 1980 when only Crabapple Creek increased compared to 1979 (Fig. 3.6). EPT richness declined again in 1981 in Bill's Branch, paralleling the trend seen for taxa richness. The consistent trend in taxa richness and EPT richness are not unexpected given that diversity metrics would be expected to be correlated, but still reinforces the possibility that the adverse effects of mining Bill's Branch watershed were still increasing in 1981. EPT richness was high in Bill's Branch by 2008 suggesting substantial recovery in the interval between sampling in 1981 and 2008.

MULTIMETRIC SCORES 1979, 1980, 1981, 2008 and 2011

Given the obvious differences in diversity metrics, it is somewhat surprising there was no significant difference in multimetric scores. The other metrics of the multimetric index dampened the differences between mined versus un-mined streams leading to the conclusion that almost all the streams are not impaired over all dates.

Tolbert et al. (1980) concluded, based on macroinvertebrate communities of streams differing in time since they were mined, that biological communities in an impacted stream should begin to show improvement within the first five years post-mining, and would continue to improve in the following years, and finally should recover to normal pre-mining conditions within 25 years if no further adverse impacts occurred. Tolbert et al. (1980) based this hypothesis on species diversity indices and abundance of aquatic insect communities of these streams which were observed to increase after 5 years post mining.

The 2008 and 2011 data shows that Green Branch and Bill's Branch scored in TDEC's not impaired category. Therefore, the results presented in this study support Tolbert's hypothesis of recovery time. The mined streams (Green and Bill's Branches) now have multimetric scores as high as the un-mined streams. Also, the most recent mining and logging activities in the Green Branch and Bill's Branch watersheds (1-3 years prior to 2008) does not appear to have had the drastic effect in the mined watersheds seen in pre-SMCRA mining operations, thus the logical conclusion is that SMCRA reclamation requirements are mitigating at least some of the adverse effects coal mining has on these headwater streams.

The decrease in multimetric score observed in Lowe Branch in 2008 may reflect the high flow conditions immediately following a spate at the time it was sampled. The richness metrics were not affected, and abundance was highest of any year, so the decline in multimetric scores seems to imply significantly different taxonomic make-up of samples resulting in lower scores for non-richness metrics. Lowe Branch experienced less flow than Crabapple Creek in the months of the extreme drought prior to its sampling. Lowe Branch was completely dried up in June prior to its sampling in July, but Crabapple Creek, while too low to sample in June, had surface flow at that time. Either or both of these differences between Lowe Branch and Crabapple Creek would be expected to adversely impact macroinvertebrate community structure and sample collection.

Macroinvertebrate abundance showed the same pattern seen for diversity metrics, generally increasing in each subsequent sample year until a peak in 2008 followed by a decline in 2011, but multimetric scores changed only slightly. The multimetric scores in Green and Bill's branches were not much lower than the un-mined streams during 1979, 1980, and 1981 and occasionally higher in 2008 and 2011, whereas the abundance in mined streams was generally much lower than that of un-mined streams in 1979, 1980, and 1981. This could indicate that even given the damage caused by the mining activity, the aquatic macroinvertebrate communities were changing to adapt to new habitats and altered stream hydrology. It does not seem that the approach of compositing four pairs of Surber samplers decreased the sensitivity of TDEC's TN Macroinvertebrate Index by inflating the multimetric scores in comparison to the "200 Pick" performed in Whitley's (2009) study. Only one of the samples of the mined streams in 1979-1981 contained more macroinvertebrates than the TDEC 200 pick range (i.e. 200±40), whereas all but one of the un-mined streams contained more macroinvertebrates than the TDEC 200 pick range, yet the multimetric scores were fairly similar between the mined and un-mined streams.

Mining operations in the watersheds of Green and Bill's branches ceased approximately four to five years prior to the 1979 sample collection, and it is very apparent that the mining operations impacted both Green and Bill's branches, with only 45 and 237 macroinvertebrates collected, respectively (Fig. 3.8). Yet the multimetric scores for Green and Bill's branches in 1979 of 18 (moderately impaired) and 40 (not impaired), respectively, do not explain the low abundance values. In addition, only 84 macroinvertebrates were collected in Crabapple Creek in 1979. Given the collection of only 45 macroinvertebrates in four pairs of composited Surber samples from Green Branch, it seems unlikely that the stream could be considered only moderately impaired. Conversely, only 84 macroinvertebrates were collected from Crabapple Creek in 1979. Macroinvertebrate abundance did increase by approximately 50% with each consecutive study, but this had little if any effect on the multimetric score since all streams, regardless of the number of macroinvertebrates collected scored in the non-impaired category (Fig. 3.7 – 3.8). This may indicate that the Tennessee Macroinvertebrate Index needs to include abundance of macroinvertebrates collected in addition to the other metrics and/or possibly some different metrics.

EFFECTS OF DROUGHT ON MACROINVERTEBRATE BIOASSESSMENTS

The effects of adverse weather conditions, such as drought, are easily seen in the analysis of abiotic factors (Fig. 3.1 – 3.4). In 1979, the yearly rainfall amount was approximately 35", followed by approximately 70" in 1980 (NOAA, 2012). The most discernible differences in the 2008 and 2011 sampling years were the flow conditions present in the streams during sampling times in 2008. Yearly rainfall amount in 2008 was 48", which is close to the amount of rainfall in 2011 (52"), however, in 2007, the yearly rainfall amount was only 35", which exacerbated the drier conditions of 2008. Low flow conditions were present during the first attempt to sample in June, resulting in a second attempt in July, which was prefaced by a large storm system passing through the area just before sampling (Whitley, 2009). Despite these extreme conditions, the only metric in the 2008 data that displays obvious deviation from other years was taxa richness (excepting abundance which was high, but is not included in the multimetric).

EFFECTS OF MINING ON MACROINVERTEBRATE COMMUNITY STRUCTURE

Some of the community structure changes observed in mined streams can be attributed to the altered hydrology and increased sedimentation caused by mining (Tolbert, 1978; Talak, 1977). Changes within these macroinvertebrate communities, especially among functional feeding groups that are vital to the processing of particulate organic matter, such as scrapers, grazers, and collectors (Schiller, 1986) can have an impact on the aquatic communities' downstream (Vannote et al., 1980).

The River Continuum Concept of Vannote et al. (1980) explains the importance of headwater streams in the conversion of allochthonous coarse particulate organic matter (CPOM; woody debris, fallen leaves, etc.) into fine particulate organic matter (FPOM). This conversion is the process by which essential particulate organic matter (POM) and energy flow into larger streams and rivers, giving rise to complex food webs that provide nutrients to aquatic life (Schiller, 1986). If these headwater streams are damaged or severely stressed, energy flow will be disrupted, resulting in shorter food chains and simpler food webs (Hogsden and Harding, 2012). These short food chains are exacerbated by intense and/or frequent stress and reduced resource availability (Hogsden and Harding, 2012).

Mine affected streams experience increases in sedimentation and embeddedness from excess run-off and erosion, which, as the sediment becomes packed into the interstitial spaces of the substrate, decreases the surface area of the substrate available to filter and control the flow of water (Schiller, 1986; Tolbert, 1978) and increases base flow and velocity of the water. Normally, headwater streams contain masses of leaf litter, accumulations of woody debris, and other detrital materials available for processing by macroinvertebrates (Schiller, 1986; Tolbert, 1978). This POM can be dislodged by major rain events, but these events normally occur only a few times annually in streams unaffected by mining. In mine affected streams, which tend to be much flashier (flashiness describes the stream's brief and rapid increases in discharge in response to precipitation events), this organic material can be dislodged more frequently, depleting the amount of POM available for processing (Schiller, 1986). Within the macroinvertebrate communities, this directly affects shredders, since they play an essential part in the process of converting coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM), but can also be detrimental to grazers, collectors, and predators as well. Grazers are adversely affected by the greatly reduced diatom abundance in streams caused by sediment deposition and scouring of surfaces by inorganic particles and collectors are adversely affected when the amounts of FPOM in the water column are diminished by and the flushing of CPOM and FPOM from the stream by more frequent high flow events. Additionally, some allochthonous carbon may be rendered inaccessible by increases in sedimentation from erosion of mining overburden. Predators, of course, will experience detrimental effects any time there is a decline in the abundance and biomass of available prey. In addition, sedimentation can cause macroinvertebrates to drift in search of a more hospitable environment (Hogsden and Harding, 2012; Schiller, 1986).

SMCRA RECLAMATION REQUIREMENTS

Samples collected in 2011 have shown that multimetric scores in the most heavily mined stream, Green Branch, has improved since the original study by Vaughan et al. (1982) (Fig. 3.7). This illustrates that SMCRA-regulated reclamation efforts are mitigating some impacts from past and present mining operations within this watershed, but lingering effects remain. For example, many pollution intolerant taxa collected by Vaughan et al. (1982) are now greatly reduced or absent when compared to the 2011 taxa list (see Appendix A). This leads to a hypothesis that while SMCRA is mitigating damage done to the aquatic communities, the composition of the macroinvertebrate communities may be changing. Ephemeroptera for example, including intolerant genera such as *Ameletus*, *Drunella*, *Ephemerella* and *Paraleptophlebia* were found in greater numbers in earlier studies (Whitley, 2009; Schiller, 1986; Vaughan et al., 1982). Patterns in community changes similar to this, including, but not limited to, taxa within Ephemeroptera have been documented in several studies concerning both surface coal mining and mountain-top removal mining effects on headwater streams (Finn, 2011; Pond, 2008; Tolbert, 1978; Talak, 1977). Specifically, Bradfield (1986) found an inverse relationship between the composition of the macroinvertebrate sample and increasing levels of water-quality constituents, resulting in a reduction of taxa from Ephemeroptera and Plecoptera, both of which have numerous pollution intolerant species (Bradfield, 1986). In contrast, Bradfield (1986) found a positive correlation between dipterans (generally a more pollution tolerant group) and decreases in water-quality (Bradfield, 1986). Talak (1977) noted that the most obvious change in taxa present in mined streams were from the orders Ephemeroptera and Trichoptera. Talak also states that immediately following the cessation of mining, all orders were affected by mining impacts, however, Ephemeroptera showed the largest initial decline and very little recovery over time (Talak, 1977). Trichoptera, while showing an initial decline, eventually seemed to recover with the passage of time (Talak, 1977). Tolbert (1978) reported lower percent composition in Ephemeroptera, Plecoptera, and Coleoptera in mined streams respective to the un-mined stream; conversely, the percent composition of Diptera was lower in the un-mined stream and higher in the mined streams. Pond et al. (2008) reported very different macroinvertebrate assemblages between mined and un-mined streams in a West Virginia study of Mountaintop Mining (MTM). They reports that entire orders of macroinvertebrates (most often Ephemeroptera) were eliminated or nearly eliminated in streams affected by MTM (Pond et al., 2008). Pond et al. (2008) also documented that mined streams contained "signature communities" dominated by both facultative and pollution tolerant taxa (Pond et al., 2008).

EVALUATION OF REFERENCE CONDITION STREAM, CRABAPPLE CREEK

Taxa Richness increased progressively from 1979 – 2011 in Crabapple Creek. A portion of this increase can be attributed to advances in taxonomic classification. For example, new genera have been recognized in the mayfly family Baetidae. During the time of Vaughan et al. (1982), taxonomic identification was limited to the visual examination of morphological characteristics only. Currently, the fields of cladistics and systematics have brought molecular analysis of DNA and precise morphological characteristics to the forefront of taxonomic classification. These techniques allow us to more accurately distinguish between certain genera of the mayfly family Baetidae. In the late 1970's, a large number of mayflies were classified under the genus *Baetis*. As technology advanced, several Baetid species' have been moved into other genera, such as *Acentrella* and *Acerpenna.* Thus, many macroinvertebrates identified by Vaughan et al. (1982) as *Baetis* would now be identified to other genera, which could be the source of the increases seen in taxa richness. Of course, it is possible that taxa richness actually did increase over this time span, but it seems unlikely that all of the observed increase was due to an actual increase in the diversity of the macroinvertebrate community. This would imply that some subtle, undetected influence had reduced diversity in the past and has since been removed, or, conversely, some subtle, undetected effect has increased diversity over time. An example of the former might be speculated as something such as an acid rain effect, while an equally speculative example of the latter might be atmospheric enrichment via nitrate deposition. Both possibilities are beyond the scope of this study to address. One can say with certainty that this increase in taxa richness does not reduce the bioassessment standard to which potentially impacted streams are compared.

FINAL CONCLUSIONS AND FUTURE CONSIDERATIONS

Although some interesting parallels between mining activities and damage to the headwater streams in the affected watersheds have been drawn during the course of this study, much more work is needed before the question of whether the mined streams are completely recovered can be addressed. Studies similar to the ones conducted by this study, Whitley (2009), Schiller (1986), and Vaughan et al. (1982) need to be implemented and carried out frequently, perhaps semi-annually, if not annually. Bioassessment every few years simply does not provide sufficient resolution to assess these streams. Based on the metric analyses in this study, there is very little evidence to support the claim that SMCRA reclamation protocols are not working. However, there are still large gaps in the current data that obscure what is occurring in these streams and watersheds during the years between studies.

Another source of variation in bioassessments results from subtle differences in methodology. Taxa richness and EPT richness are sensitive to the size of surface area sampled. Whitley (2009) performed bioassessments on each pair of Surber samples and calculated the mean score of the eight paired Surber samples. Thus, Whitley's bioassessments were based on sampled areas of 0.2 m^2 (approximately 2 ft^2). This method produced significantly lower diversity metric scores and lower overall multimetric scores in her bioassessments. In TDEC's SQKicknet protocol, the "four composited kick nets with a 200 Pick" sample approximately twice the surface area of Whitley's study and half the streambed surface area sampled by four paired Surber samples (eight chosen riffle areas per stream) used to conduct bioassessments in this study. Despite the greater surface area of kicknet samples compared to Surber samples in Whitley's study, she obtained similar bioassessments for both. This suggests Surber samplers may be a more efficient collecting device than kicknets. Another variable that will affect richness metrics is simply the number of macroinvertebrates in the sample. A "200 Pick" sample usually contains fewer macroinvertebrates than in 4 paired Surber samples composited. Fewer total macroinvertebrates identified usually results in lower taxa richness and lower EPT richness. Subsequent studies should compare bioassessments based on similar streambed area sampled and explore the effect of bioassessments of composited Surber samples incorporating the "200 Pick" methodology of the TDEC protocol.

Numerous studies over the last three decades (Chambers et al., 2014; Murphy et al., 2012; Bernhardt and Palmer, 2011; Lindberg et al., 2011; Pond et al., 2008; Freund et al., 2007; Dickens et al., 1989) have repeatedly shown that the correlation between mining activities and damage to headwater streams not only exists, but also that the affected streams have not completely recovered to their pre-mining state even with the reclamation requirements put in place by SMCRA.

Freund et al. (2007) sought to identify specific chemical conditions or chemical constituents that were directly responsible for detrimental effects on macroinvertebrate and fish communities. The study was conducted in West Virginia in the Cheat River watershed. Multiple indices were used in the analysis: West Virginia Stream Condition Index (WV-SCI) for macroinvertebrates; Mid-Atlantic Highlands Index of Biotic Integrity (MAH-IBI) for fish; and Principle Components Analysis (PCA) for chemical constituents.

Their results indicated that West Virginia's water-quality standards have been set too high. Impairment to both macroinvertebrates and fish occurred at much lower levels than the standard. Final conclusions revealed that "biological assemblages respond to a suite of chemical constituents rather than a single critical toxin" (Freund et al., 2007). Freund et al. (2007) stated that meeting water quality standards will not result in full recovery of mining impacted streams. This last statement parallels the results of this study. All of the streams in this study, mined and un-mined displayed multimetric scores determined by TDEC's TN Macroinvertebrate Index as being "not impaired". The macroinvertebrate abundance analysis demonstrated that revisiting and re-examining the criteria that determine a stream's multimetric score is necessary. Green Branch is the most heavily mined stream in this study, and with a total of nine macroinvertebrates collected in four paired Surber samples in 1980, it scored in the slightly impaired category (30). This seems to be an unlikely result for a stream with so few individuals. Strict adherence to TDEC SQKicknet protocol would have required additional sample collection sufficient to yield a 200 pick, but this would have required approximately 50 Surber samples based on projecting the total number of macroinvertebrates, 49, collected in the eight paired Surber samples that day.

Land use has also been the subject of several studies. Chambers et al. (2014) states that "basins that differed most from the minimally affected reference condition were those basins in which coal mining was the dominant non-forest land use...and basins in which agriculture was important were more similar to the reference condition". That study was also conducted in West Virginia in the Kanawha River Basin.

My investigation focused on an area in the Cumberland Mountains in which the primary land uses are coal mining and to a smaller extent, agriculture. The steepness of the terrain and lack of paved roads in the area make industrial and urban use next to impossible. Thus, the conclusion can be made that most anthropogenic impacts to the headwater study streams are the result of extensive mining activity. Given the conclusions of Freund et al. (2007) and Tolbert's (1978) hypothesis, which was that mined streams would experience complete recovery after a period of approximately 25 years, there seems to be an evolving insight into what is actually happening in the stream. The results of multimetric analysis from the streams in the Cumberland Mountains appear to uphold Tolbert's (1978) hypothesis. However, Freund et al. (2007) reveals that many of the standards in use today are not sensitive enough to pinpoint the exact level of impairment necessary to cause damage to the aquatic organisms in the mined streams.

Coal mining permanently alters the hydrology, chemistry, and biology of streams as discussed in previous chapters, but what is not always considered is the effect of this damage to downstream areas and even into the river system itself (Bernhardt and Palmer, 2011). The food webs associated with aquatic ecosystems can be large and very complex particularly if mining is prevalent in the watershed. Whatever damage is done to headwater-stream macroinvertebrates and fishes propagates downstream to larger organisms and eventually into the human food web (Bernhardt and Palmer, 2011). For example, if a macroinvertebrate absorbs a certain amount of a chemical constituent (e.g., as a heavy metal), that constituent is passed on to the small fishes that eat the macroinvertebrates, then to the larger fishes that eat the smaller fishes, and continues to be passed to larger and more complex organisms. The original chemical constituent may not be toxic to macroinvertebrates or fishes, but it may be toxic to larger animals and humans. What was perceived to be a localized problem has now become a systemic condition through bioaccumulation.

In fulfilling the objectives of this study, analysis and comparison of biotic metrics and abiotic measurements were completed among all years (1979, 1980, 1981, 2008, and 2011). The results did show significant differences between mined and un-mined streams in the following analyses: conductivity, EPT richness and taxa richness; however, multimetric scores indicate that all mined streams now score in TDEC's non-impaired category, which leads to the conclusion that the mined streams have recovered from adverse impacts caused by long-term mining operations in and near the streams' watersheds. The conflicting results regarding the individual metrics could indicate that TDEC's Tennessee Macroinvertebrate Index needs to be revised in order to be more sensitive to the subtle changes seen in headwater streams. TDEC's multimetric protocol was originally developed to pinpoint larger sources of pollution such as point-source pollution from industrial waste and non-point source pollution such as damage from agricultural practices; thus, the conclusions of this study are that revisions to TDEC protocols, specifically concerning headwater streams, need to be addressed. Some deviations were noted in Whitley's (2009) study as a result of the extreme drought conditions experienced in 2008; however, the largest impact of the drought seems to be the late collection of samples forced by insufficient flow in un-mined streams. Based on the multimetric scores analyzed in this study, (all mined streams scored in TDEC's non-impaired category) SMCRA reclamation protocols are in fact working. Despite the significant differences in individual metrics (taxa richness, EPT richness, and macroinvertebrate abundance), there is not enough evidence to support the claim that reclamation practices are not fulfilling their requirements. Final conclusions for this study are that on-going studies, reassessment of ecoregions, and revisions of the criteria used to assess the health of the macroinvertebrate community need to be addressed.

"Streams are the gutters down which flow the ruins of continents" Leopold et al. (1964).

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Appendix A: Taxa List - 2011

Coleoptera	
Psephenidae	
Psephenus herricki	107
Ectopria	18
Microcylloepus (adult)	2
Stenelmis (adult)	4
Microcylloepus (larvae)	3
Stenelmis (larvae)	19
Decapoda	
Cambaridae	
Orconectes	2
Cambarus	1
Diptera	
Psychodidae	2
pupae	2
Chironomidae	311
Chaoboridae	
Chaoborus	1
Simulidae	1
Prosimulium	7
Simulium	5
Limoniinae (subfamily Tipullidae)	
Antocha	4
Pedicia	8
Cryptolabis	8
Tipulidae (pupa)	1
Brachypremna dispellens	3
Hexatoma	27
Leptotarsus	12
Tipula	4
Ephemeroptera	
Ameletidae	
Ameletus	55
Baetidae	7
Heterocleon	8
Caenis	15
Ephemerellidae	
Drunella	2
Ephemerella	17
Attenella	2
Serratella	3
Leptophlebiidae	
Habrophlebia	1
Paraleptophlebia	78
Leptophlebia	43
Isonychiidae	
Isonychia	6
Ephemeridae	
Ephemera	5
Neoephemeridae	
Neoephemera	2
Heptageniidae	
Cinygmula	43
Epeorus	59
Heptagenia	4
Maccaffertium	8
Stenacron	2
Stenonema femoratum	9
Hemiptera	
Veliidae	
Microvelia	1
Isopoda	
Asellidae	
Lirceus	20
Megaloptera	
Corydalidae	
Nigronia	1
Sialidae	
Sialis	1
Odonata	
Gomphidae	2
Gomphus	1
Lanthus	1
Oligochaeta	17
Platyhelminthes/Tricladida	2
Plecoptera	19
Perlidae	0
Acroneuria	29

Beloneuria	2
Chloroperlidae	
Suwallia	47
Sweltsa	8
Leuctridae	14
Leuctra	4
Nemouridae	10
Amphinemura	6
Perlodidae	
Malirekus	1
Isoperla	5
Yugus	1
Peltoperlidae	18
Viehoperla ada	1
Trichoptera	1
Brachycentridae	
Brachycentrus	1
Glossosomatidae	
Glossosoma	1
Goeridae	
Pupa	1
Hydropsychidae	
Cheumatopsyche	5
Hydropsyche	1
Pupa	1
Diplectrona	23
Lepidostomatidae	
Pupa	1
Limnephilidae	
Pycnopsyche	1
Molannidae	
Pupa	2
Philopotamidae	
Chimarra	0
Wormaldia	2
Psychomyiidae	
Lype	1
Polycentropodidae	
Neureclipsis	2
Polycentropus	3
Ryacophilidae	
Ryacophila	1
Uenoidae	
Neophylax	13

LOWE BRANCH

Coleoptera	
Psephenidae	
Psephenus herricki	12
Ectopria	6
Elmidae	
Gonielmis dietrichi (larvae)	1
Optioservus (larvae)	1
Curculionidae	
Bagous	6
Diptera	10
Chironomidae	115
Simulidae	
Prosimulium	1
Limoniinae (subfamily Tipullidae)	
Pedicia	1
Cryptolabis	4
Tipulidae (pupa)	
Hexatoma	6
Ephemeroptera	
Ameletidae	
Ameletus	11
Baetidae	
Baetis	12
Centroptilum	2
Heterocleon	6
Ephemerellidae	
Ephemerella	23
Serratella	3
Leptophlebiidae	
Paraleptophlebia	50
Ephemeridae	
Ephemera	1
Heptageniidae	
Cinygmula	28
Epeorus	26
Maccaffertium	6
Stenacron	4
Stenonema femoratum	6
Annelida	1
Lepidoptera	
Crambidae	7
Oligochaeta	2
Plecoptera	
Perlidae	
Acroneuria	4
Eccoptura	1
Chloroperlidae	
Suwallia	34
Sweltsa	7
Leuctridae	
Leuctra	4
Nemouridae	
Amphinemura	2
Perlodidae	1
Malirekus	2
Diploperla	1
Peltoperlidae	8
Peltoperla	1
Trichoptera	
Hydropsychidae	
Cheumatopsyche	6
Ceratopsyche	3
Diplectrona	5
Ryacophilidae	
Rhyacophila	3
Uenoidae	
Neophylax	2

BILL'S BRANCH

Coleoptera	
Psephenidae	
Psephenus herricki	13
Ectopria	5
Dytiscidae	
Hydaticus	1
Elmidae	
Microcylloepus (adult)	1
Stenelmis (adult)	1
Stenelmis (larvae)	1
Diptera	1
Psychodidae	
pupae	3
Chironomidae	55
Limoniinae (subfamily Tipullidae)	
Cryptolabis	5
Tipulidae (pupa)	3
Hexatoma	5
Tipula	1
Ephemeroptera	
Ameletidae	
Ameletus	8
Baetidae	
Baetis	16
Centroptilum	6
Heterocleon	2
Ephemerellidae	
Ephemerella	1
Serratella	1
Leptophlebiidae	
Paraleptophlebia	83
Heptageniidae	
Cinygmula	131
Epeorus	4
Maccaffertium	5
Stenacron	4
Stenonema femoratum	2
Annelida	1
Lepidoptera	
Crambidae	1
Oligochaeta	2
Plecoptera	1
Perlidae	
Acroneuria	2
Chloroperlidae	
Suwallia	85
Sweltsa	5
Leuctridae	
Leuctra	1
Nemouridae	
Amphinemura	17
Perlodidae	
Malirekus	1
Trichoptera	
Hydropsychidae	
Ceratopsyche	1
Ryacophilidae	
Ryacophila	1
Uenoidae	
Neophylax	1

GREEN BRANCH

Coleoptera	
Psephenidae	
Psephenus herricki	9
Ectopria	3
Diptera	4
Chironomidae	32
Simulidae	
Prosimulium	3
Simulium	15
Limoniinae (subfamily Tipullidae)	
Cryptolabis	1
Tipulidae (pupa)	4
Hexatoma	2
Ephemeroptera	
Ameletidae	
Ameletus	8
Baetidae	
Callibaetis	1
Baetis	27
Centroptilum	1
Heterocleon	9
Ephemerellidae	
Drunella	1
Ephemerella	7
Leptophlebiidae	
Paraleptophlebia	6
Leptophlebia	2
Heptageniidae	
Cinygmula	157
Epeorus	47
Maccaffertium	4
Stenacron	1
Stenonema femoratum	5
Lepidoptera	
Crambidae	3
Oligochaeta	1
Plecoptera	
Perlidae	
Acroneuria	1
Chloroperlidae	2
Suwallia	89
Sweltsa	8
Alloperla	5
Leuctridae	11
Leuctra	1
Nemouridae	
Amphinemura	31
Perlodidae	
Malirekus	1
Diploperla	1
Isoperla	5
Peltoperlidae	1
Peltoperla	1
Trichoptera	
Hydropsychidae	
Cheumatopsyche	2
Ceratopsyche	4
Diplectrona	2
Polycentropodidae	
Polycentropus	1

Appendix B: Biometrics 1979-2011

Biometrics	1979				1980				1981				2008				2011			
	Green	Bills	Lowe	Crabapple	Green	Bills	Lowe	Crabapple	Green	Bills	Lowe	Crabapple	Green	Bills	Lowe	Crabapple	Green	Bills	Lowe	Crabapple
Total Individuals	45	237	366	84	9	374	502	325	57	110	970	535	1323	1251	817	1590	522	474	432	1172
EPT abundance	18	197	272	50	8	357	425	303	54	107	785	395	819	688	249	758	446	378	262	594
Olig. & Chiro. Abund.	26	11	62	8	1	10	24	10	3	2	45	31	255	369	273	526	32	55	115	311
abundance of Tolerants	26	33	73	24	1	15	59	18	3	4	143	76	524	474	393	674	44	74	137	449
clinger abund.	19	222	298	74	7	363	477	312	54	108	903	500	1019	801	433	1002	478	403	294	751
Tennessee Macroinvertebrate Index Metrics																				
Taxa Richness	7	30	26	13	4	16	24	23	6	13	31	31	47	44	54	53	38	32	39	69
EPT Richness	6	23	22	10	4	14	18	17	6	12	20	20	30	27	30	28	30	22	29	49
% EPT	40	83	74	60	89	95	85	93	95	97	81	74	62	55	30	48	85	80	61	51
% OC	58	5	17	10	11	3	5	3	5	2	5	6	19	29	33	33	6	12	27	27
NCBI	4	2	2	3	2	2	1	2	1	2	2	3	3	3	4	3	2	2	3	4
% NUTOL	58	14	20	29	11	4	12	6	5	4	15	14	40	38	48	42	8	16	32	38
% Clingers	42	94	81	88	78	97	95	96	95	98	93	93	77	64	53	63	92	85	68	64
Tennessee Macroinvertebrate Index Metric Scores																				
Taxa Richness	0	4	4	2	0	2	4	4	0	2	4	4	6	6	6	6	6	6	6	6
EPT Richness	2	6	6	4	0	4	6	6	2	4	6	6	6	6	6	6	6	6	6	6
% EPT	4	6	6	4	6	6	6	6	6	6	6	6	6	4	2	4	6	6	6	4
% OC	2	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6
NCBI	4	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6
% NUTOL	4	6	6	6	6	6	6	6	6	6	6	6	4	4	4	4	6	6	6	4
% Clingers	2	6	6	6	6	6	6	6	6	6	6	6	6	6	2	6	6	6	6	6

multimetric score	18	40	40	34	30	36	40	40	32	36	40	40	40	38	28	38	42	42	42	38
Bioassessment	MI	NI	NI	NI	SI	NI	NI	NI	SI	NI	NI	NI	NI	NI	SI	NI	NI	NI	NI	NI

NI = not impaired, SI = slightly impaired, MI = moderately impaired

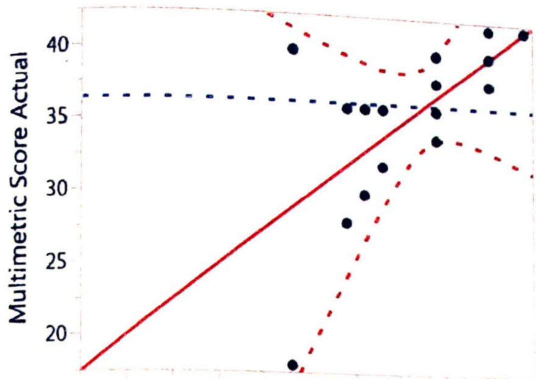
Appendix C: JMP Analyses

MULTIMETRIC ANALYSIS: MINED VS. UN-MINED STREAMS

Response Multimetric Score

Whole Model

Actual by Predicted Plot



Multimetric Score Predicted P=0.4385 RSq=0.50
RMSE=5.7271

Summary of Fit

RSquare	0.497549
RSquare Adj	0.045343
Root Mean Square Error	5.727128
Mean of Response	36.4
Observations (or Sum Wgts)	20

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	324.80000	36.0889	1.1003
Error	10	328.00000	32.8000	Prob > F
C. Total	19	652.80000		0.4385

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	36.4	1.280625	28.42	<.0001*
Mining Status[mined]	-1.4	1.280625	-1.09	0.2999
Mining Status[mined]:Year Sampled[1979]	-6	3.622154	-1.66	0.1286
Mining Status[mined]:Year Sampled[1980]	-2	3.622154	-0.55	0.5930
Mining Status[mined]:Year Sampled[1981]	-1	3.622154	-0.28	0.7881
Mining Status[mined]:Year Sampled[2008]	2	3.622154	0.55	0.5930
Mining Status[un-mined]:Year Sampled[1979]	-0.8	3.622154	-0.22	0.8296
Mining Status[un-mined]:Year Sampled[1980]	2.2	3.622154	0.61	0.5571
Mining Status[un-mined]:Year Sampled[1981]	2.2	3.622154	0.61	0.5571
Mining Status[un-mined]:Year Sampled[2008]	-5.8	3.622154	-1.60	0.1404

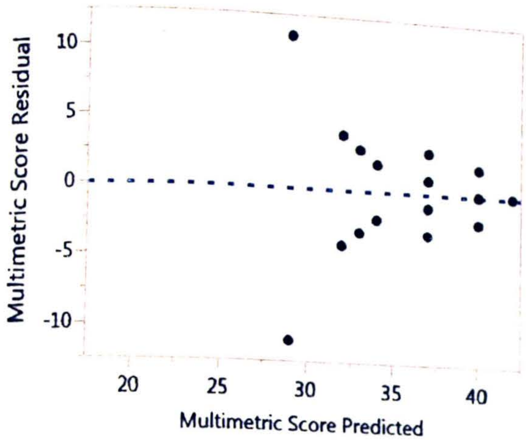
Response Multimetric Score

Whole Model

Effect Tests

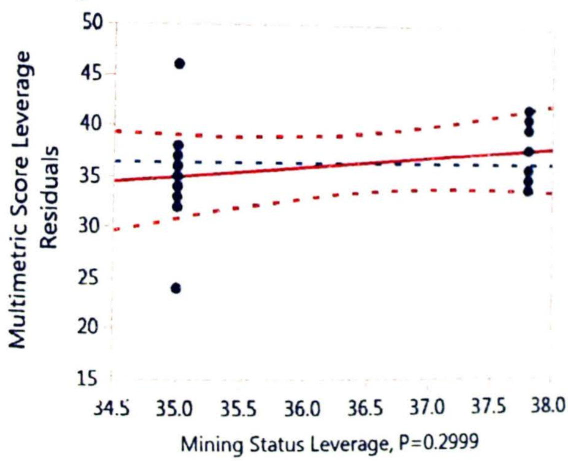
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Mining Status	1	1	39.20000	1.1951	0.2999
Year Sampled[Mining Status]	8	8	285.60000	1.0884	0.4411

Residual by Predicted Plot



Mining Status

Leverage Plot



Least Squares Means Table

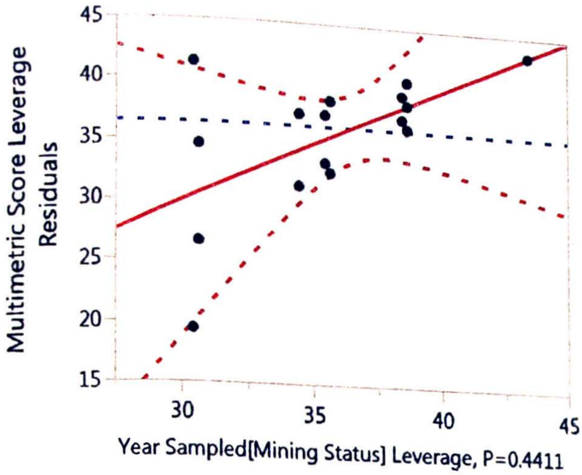
Level	Least Sq Mean	Std Error	Mean
mined	35.000000	1.8110770	35.0000
un-mined	37.800000	1.8110770	37.8000

Year Sampled[Mining Status]

Response Multimetric Score

Year Sampled[Mining Status]

Leverage Plot



Least Squares Means Table

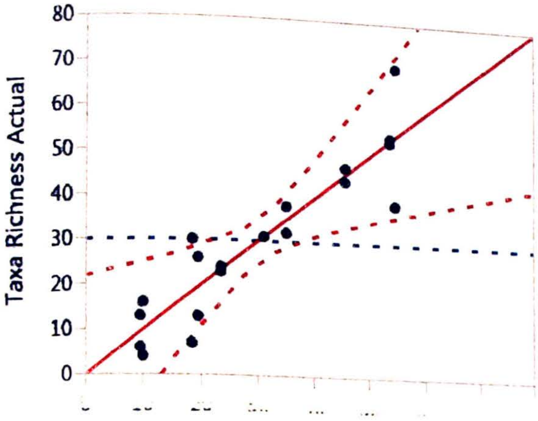
Level	Least Sq Mean	Std Error
[mined]1979	29.000000	4.0496913
[mined]1980	33.000000	4.0496913
[mined]1981	34.000000	4.0496913
[mined]2008	37.000000	4.0496913
[mined]2011	42.000000	4.0496913
[un-mined]1979	37.000000	4.0496913
[un-mined]1980	40.000000	4.0496913
[un-mined]1981	40.000000	4.0496913
[un-mined]2008	32.000000	4.0496913
[un-mined]2011	40.000000	4.0496913

TAXA RICHNESS ANALYSIS: MINED VS. UN-MINED STREAMS

Response Taxa Richness

Whole Model

Actual by Predicted Plot



Summary of Fit

RSquare	0.84167
RSquare Adj	0.699172
Root Mean Square Error	9.744229
Mean of Response	30.05
Observations (or Sum Wgts)	20

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	5047.4500	560.828	5.9066
Error	10	949.5000	94.950	Prob > F
C. Total	19	5996.9500		0.0052*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	30.05	2.178876	13.79	<.0001*
Mining Status[Mined]	-6.35	2.178876	-2.91	0.0154*
Mining Status[Mined]:Year Sampled[1979]	-5.2	6.162792	-0.84	0.4185
Mining Status[Mined]:Year Sampled[1980]	-13.7	6.162792	-2.22	0.0504
Mining Status[Mined]:Year Sampled[1981]	-14.2	6.162792	-2.30	0.0439*
Mining Status[Mined]:Year Sampled[2008]	21.8	6.162792	3.54	0.0054*
Mining Status[Un-mined]:Year Sampled[1979]	-16.9	6.162792	-2.74	0.0208*
Mining Status[Un-mined]:Year Sampled[1980]	-12.9	6.162792	-2.09	0.0628
Mining Status[Un-mined]:Year Sampled[1981]	-5.4	6.162792	-0.88	0.4015
Mining Status[Un-mined]:Year Sampled[2008]	17.1	6.162792	2.77	0.0196*

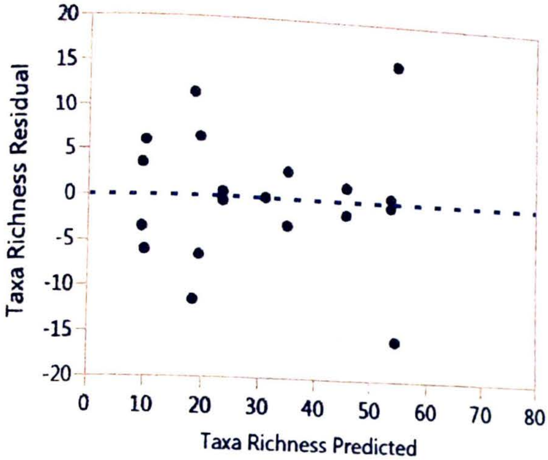
Response Taxa Richness

Whole Model

Effect Tests

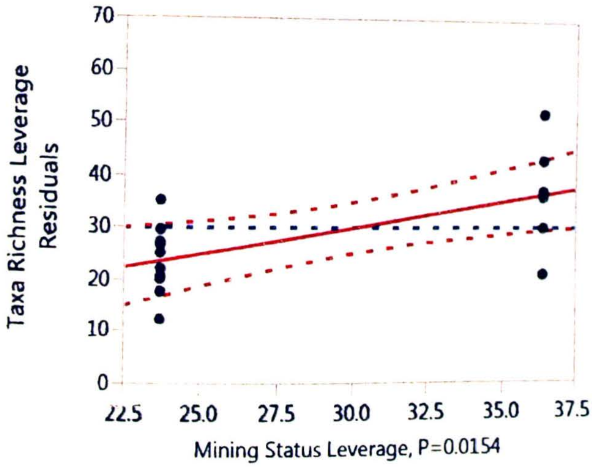
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Mining Status	1	1	806.4500	8.4934	0.0154*
Year Sampled[Mining Status]	8	8	4241.0000	5.5832	0.0070*

Residual by Predicted Plot



Mining Status

Leverage Plot



Least Squares Means Table

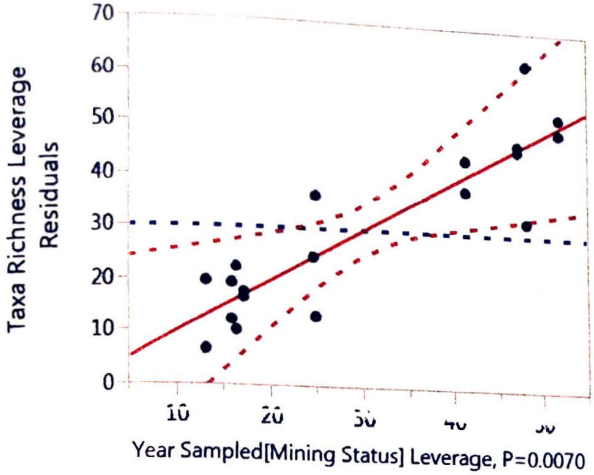
Level	Least Sq Mean	Std Error	Mean
Mined	23.700000	3.0813958	23.7000
Un-mined	36.400000	3.0813958	36.4000

Year Sampled[Mining Status]

Response Taxa Richness

Year Sampled[Mining Status]

Leverage Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error
[Mined]1979	18.500000	6.8902104
[Mined]1980	10.000000	6.8902104
[Mined]1981	9.500000	6.8902104
[Mined]2008	45.500000	6.8902104
[Mined]2011	35.000000	6.8902104
[Un-mined]1979	19.500000	6.8902104
[Un-mined]1980	23.500000	6.8902104
[Un-mined]1981	31.000000	6.8902104
[Un-mined]2008	53.500000	6.8902104
[Un-mined]2011	54.500000	6.8902104

LSMeans Differences Tukey HSD

α= 0.050 Q= 3.95864

Response Taxa Richness

Year Sampled[Mining Status]

LSMeans Differences Tukey HSD

LSMean[i] \ LSMean[j] Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	[Mined] 1979	[Mined] 1980	[Mined] 1981	[Mined] 2008	[Mined] 2011	[Un-mined]1979	[Un-mined]1980	[Un-mined]1981	[Un-mined]2008	[Un-mined]2011
[Mined]1979	0	8.5	9	-27	-16.5	-1	-5	-12.5	-35	-36
	0	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423
	0	-30.074	-29.574	-65.574	-55.074	-39.574	-43.574	-51.074	-73.574	-74.574
	0	47.0739	47.5739	11.5739	22.0739	37.5739	33.5739	26.0739	3.57389	2.57389
[Mined]1980	-8.5	0	0.5	-35.5	-25	-9.5	-13.5	-21	-43.5	-44.5
	9.74423	0	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423
	-47.074	0	-38.074	-74.074	-63.574	-48.074	-52.074	-59.574	-82.074	-83.074
	30.0739	0	39.0739	3.07389	13.5739	29.0739	25.0739	17.5739	-4.9261	-5.9261
[Mined]1981	-9	-0.5	0	-36	-25.5	-10	-14	-21.5	-44	-45
	9.74423	9.74423	0	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423
	-47.574	-39.074	0	-74.574	-64.074	-48.574	-52.574	-60.074	-82.574	-83.574
	29.5739	38.0739	0	2.57389	13.0739	28.5739	24.5739	17.0739	-5.4261	-6.4261
[Mined]2008	27	35.5	36	0	10.5	26	22	14.5	-8	-9
	9.74423	9.74423	9.74423	0	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423
	-11.574	-3.0739	-2.5739	0	-28.074	-12.574	-16.574	-24.074	-46.574	-47.574
	65.5739	74.0739	74.5739	0	49.0739	64.5739	60.5739	53.0739	30.5739	29.5739
[Mined]2011	16.5	25	25.5	-10.5	0	15.5	11.5	4	-18.5	-19.5
	9.74423	9.74423	9.74423	9.74423	0	9.74423	9.74423	9.74423	9.74423	9.74423
	-22.074	-13.574	-13.074	-49.074	0	-23.074	-27.074	-34.574	-57.074	-58.074
	55.0739	63.5739	64.0739	28.0739	0	54.0739	50.0739	42.5739	20.0739	19.0739
[Un-mined]1979	1	9.5	10	-26	-15.5	0	-4	-11.5	-34	-35
	9.74423	9.74423	9.74423	9.74423	9.74423	0	9.74423	9.74423	9.74423	9.74423
	-37.574	-29.074	-28.574	-64.574	-54.074	0	-42.574	-50.074	-72.574	-73.574
	39.5739	48.0739	48.5739	12.5739	23.0739	0	34.5739	27.0739	4.57389	3.57389
[Un-mined]1980	5	13.5	14	-22	-11.5	4	0	-7.5	-30	-31
	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	0	9.74423	9.74423	9.74423
	-33.574	-25.074	-24.574	-60.574	-50.074	-34.574	0	-46.074	-68.574	-69.574
	43.5739	52.0739	52.5739	16.5739	27.0739	42.5739	0	31.0739	8.57389	7.57389
[Un-mined]1981	12.5	21	21.5	-14.5	-4	11.5	7.5	0	-22.5	-23.5
	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	0	9.74423	9.74423
	-26.074	-17.574	-17.074	-53.074	-42.574	-27.074	-31.074	0	-61.074	-62.074
	51.0739	59.5739	60.0739	24.0739	34.5739	50.0739	46.0739	0	16.0739	15.0739
[Un-mined]2008	35	43.5	44	8	18.5	34	30	22.5	0	-1
	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	0	9.74423
	-3.5739	4.92611	5.42611	-30.574	-20.074	-4.5739	-8.5739	-16.074	0	-39.574
	73.5739	82.0739	82.5739	46.5739	57.0739	72.5739	68.5739	61.0739	0	37.5739
[Un-mined]2011	36	44.5	45	9	19.5	35	31	23.5	1	0
	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	9.74423	0
	-2.5739	5.92611	6.42611	-29.574	-19.074	-3.5739	-7.5739	-15.074	-37.574	0
	74.5739	83.0739	83.5739	47.5739	58.0739	73.5739	69.5739	62.0739	39.5739	0

Response Taxa Richness

Year Sampled[Mining Status]

LSMeans Differences Tukey HSD

Level			Least Sq Mean
[Un-mined]2011	A		54.500000
[Un-mined]2008	A		53.500000
[Mined]2008	A	B	45.500000
[Mined]2011	A	B	35.000000
[Un-mined]1981	A	B	31.000000
[Un-mined]1980	A	B	23.500000
[Un-mined]1979	A	B	19.500000
[Mined]1979	A	B	18.500000
[Mined]1980		B	10.000000
[Mined]1981		B	9.500000

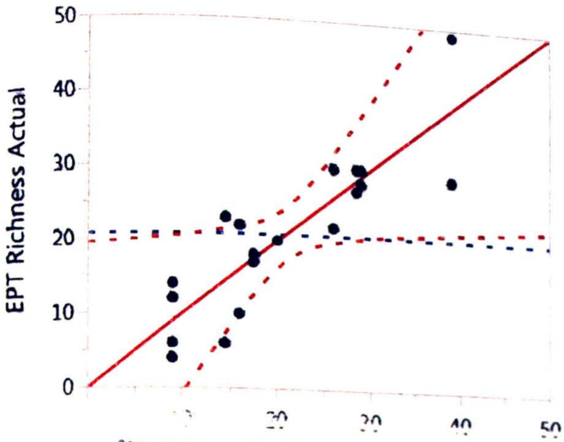
Levels not connected by same letter are significantly different.

EPT RICHNESS ANALYSIS: MINED VS. UN-MINED STREAMS

Response EPT Richness

Whole Model

Actual by Predicted Plot



Summary of Fit

RSquare	0.761889
RSquare Adj	0.547588
Root Mean Square Error	7.23533
Mean of Response	20.85
Observations (or Sum Wgts)	20

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	1675.0500	186.117	3.5552
Error	10	523.5000	52.350	Prob > F
C. Total	19	2198.5500		0.0304*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	20.85	1.617869	12.89	<.0001*
Mining Status[mined]:Year Sampled[1979]	-2.9	4.576024	-0.63	0.5405
Mining Status[mined]:Year Sampled[1980]	-8.4	4.576024	-1.84	0.0963
Mining Status[mined]:Year Sampled[1981]	-8.4	4.576024	-1.84	0.0963
Mining Status[mined]:Year Sampled[2008]	11.1	4.576024	2.43	0.0357*
Mining Status[un-mined]:Year Sampled[1979]	-8.3	4.576024	-1.81	0.0998
Mining Status[un-mined]:Year Sampled[1980]	-6.8	4.576024	-1.49	0.1681
Mining Status[un-mined]:Year Sampled[1981]	-4.3	4.576024	-0.94	0.3695
Mining Status[un-mined]:Year Sampled[2008]	4.7	4.576024	1.03	0.3286
Mining Status[mined]	-3.45	1.617869	-2.13	0.0588

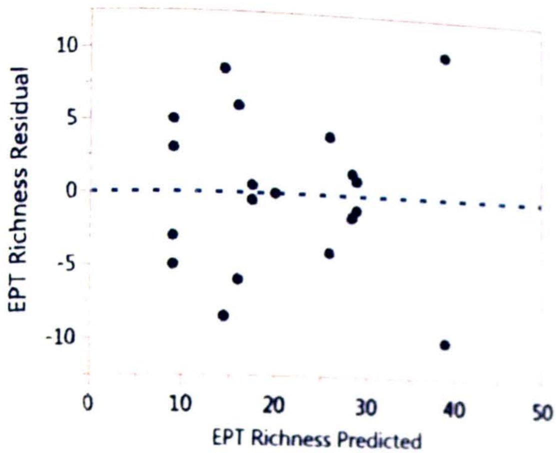
Response EPT Richness

Whole Model

Effect Tests

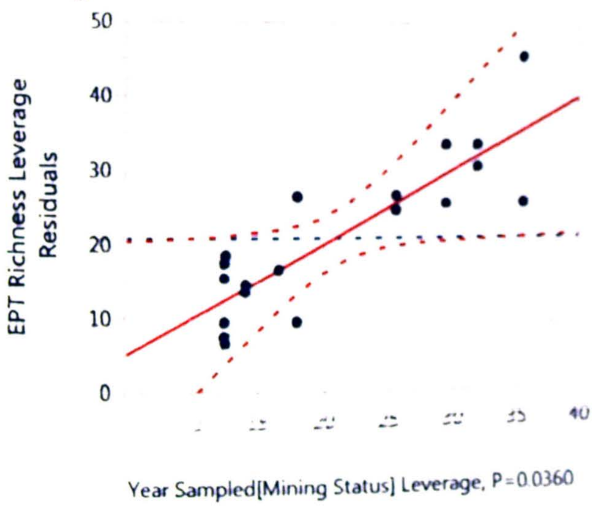
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Year Sampled[Mining Status]	8	8	1437.0000	3.4312	0.0360*
Mining Status	1	1	238.0500	4.5473	0.0588

Residual by Predicted Plot



Year Sampled[Mining Status]

Leverage Plot



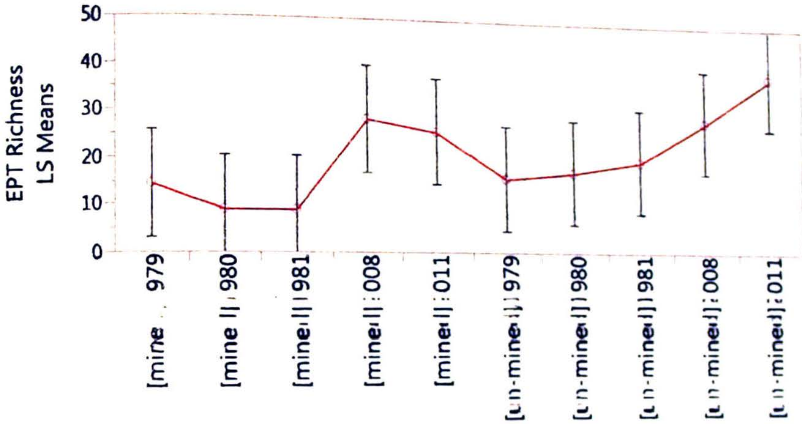
Response EPT Richness

Year Sampled[Mining Status]

Least Squares Means Table

Level	Least Sq Mean	Std Error
[mined]1979	14.500000	5.1161509
[mined]1980	9.000000	5.1161509
[mined]1981	9.000000	5.1161509
[mined]2008	28.500000	5.1161509
[mined]2011	26.000000	5.1161509
[un-mined]1979	16.000000	5.1161509
[un-mined]1980	17.500000	5.1161509
[un-mined]1981	20.000000	5.1161509
[un-mined]2008	29.000000	5.1161509
[un-mined]2011	39.000000	5.1161509

LS Means Plot



LSMeans Differences Tukey HSD

α= 0.050 Q= 3.95864

Response EPT Richness

Year Sampled[Mining Status]

LSMeans Differences Tukey HSD

LSMean[j]

LSMean[i]	Mean(i)-Mean(j) / Std Err Dif / Lower CL Dif / Upper CL Dif	[mined]1979	[mined]1980	[mined]1981	[mined]2008	[mined]2011	[un-mined]1979	[un-mined]1980	[un-mined]1981	[un-mined]2008	[un-mined]2011
[mined]1979	Mean(i)-Mean(j)	0	5.5	5.5	-14	-11.5	-1.5	-3	-5.5	-14.5	-24.5
	Std Err Dif	0	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533
	Lower CL Dif	0	-23.142	-23.142	-42.642	-40.142	-30.142	-31.642	-34.142	-43.142	-53.142
	Upper CL Dif	0	34.1421	34.1421	14.6421	17.1421	27.1421	25.6421	23.1421	14.1421	4.14207
[mined]1980	Mean(i)-Mean(j)	-5.5	0	0	-19.5	-17	-7	-8.5	-11	-20	-30
	Std Err Dif	7.23533	0	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533
	Lower CL Dif	-34.142	0	-28.642	-48.142	-45.642	-35.642	-37.142	-39.642	-48.642	-58.642
	Upper CL Dif	23.1421	0	28.6421	9.14207	11.6421	21.6421	20.1421	17.6421	8.64207	-1.3579
[mined]1981	Mean(i)-Mean(j)	-5.5	0	0	-19.5	-17	-7	-8.5	-11	-20	-30
	Std Err Dif	7.23533	7.23533	0	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533
	Lower CL Dif	-34.142	-28.642	0	-48.142	-45.642	-35.642	-37.142	-39.642	-48.642	-58.642
	Upper CL Dif	23.1421	28.6421	0	9.14207	11.6421	21.6421	20.1421	17.6421	8.64207	-1.3579
[mined]2008	Mean(i)-Mean(j)	14	19.5	19.5	0	2.5	12.5	11	8.5	-0.5	-10.5
	Std Err Dif	7.23533	7.23533	7.23533	0	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533
	Lower CL Dif	-14.642	-9.1421	-9.1421	0	-26.142	-16.142	-17.642	-20.142	-29.142	-39.142
	Upper CL Dif	42.6421	48.1421	48.1421	0	31.1421	41.1421	39.6421	37.1421	28.1421	18.1421
[mined]2011	Mean(i)-Mean(j)	11.5	17	17	-2.5	0	10	8.5	6	-3	-13
	Std Err Dif	7.23533	7.23533	7.23533	7.23533	0	7.23533	7.23533	7.23533	7.23533	7.23533
	Lower CL Dif	-17.142	-11.642	-11.642	-31.142	0	-18.642	-20.142	-22.642	-31.642	-41.642
	Upper CL Dif	40.1421	45.6421	45.6421	26.1421	0	38.6421	37.1421	34.6421	25.6421	15.6421
[un-mined]1979	Mean(i)-Mean(j)	1.5	7	7	-12.5	-10	0	-1.5	-4	-13	-23
	Std Err Dif	7.23533	7.23533	7.23533	7.23533	7.23533	0	7.23533	7.23533	7.23533	7.23533
	Lower CL Dif	-27.142	-21.642	-21.642	-41.142	-38.642	0	-30.142	-32.642	-41.642	-51.642
	Upper CL Dif	30.1421	35.6421	35.6421	16.1421	18.6421	0	27.1421	24.6421	15.6421	5.64207
[un-mined]1980	Mean(i)-Mean(j)	3	8.5	8.5	-11	-8.5	1.5	0	-2.5	-11.5	-21.5
	Std Err Dif	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	0	7.23533	7.23533	7.23533
	Lower CL Dif	-25.642	-20.142	-20.142	-39.642	-37.142	-27.142	0	-31.142	-40.142	-50.142
	Upper CL Dif	31.6421	37.1421	37.1421	17.6421	20.1421	30.1421	0	26.1421	17.1421	7.14207
[un-mined]1981	Mean(i)-Mean(j)	5.5	11	11	-8.5	-6	4	2.5	0	-9	-19
	Std Err Dif	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	0	7.23533	7.23533
	Lower CL Dif	-23.142	-17.642	-17.642	-37.142	-34.642	-24.642	-26.142	0	-37.642	-47.642
	Upper CL Dif	34.1421	39.6421	39.6421	20.1421	22.6421	32.6421	31.1421	0	19.6421	9.64207
[un-mined]2008	Mean(i)-Mean(j)	14.5	20	20	0.5	3	13	11.5	9	0	-10
	Std Err Dif	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	0	7.23533
	Lower CL Dif	-14.142	-8.6421	-8.6421	-28.142	-25.642	-15.642	-17.142	-19.642	0	-38.642
	Upper CL Dif	43.1421	48.6421	48.6421	29.1421	31.6421	41.6421	40.1421	37.6421	0	18.6421
[un-mined]2011	Mean(i)-Mean(j)	24.5	30	30	10.5	13	23	21.5	19	10	0
	Std Err Dif	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	7.23533	0
	Lower CL Dif	-4.1421	1.35793	1.35793	-18.142	-15.642	-5.6421	-7.1421	-9.6421	-18.642	0
	Upper CL Dif	53.1421	58.6421	58.6421	39.1421	41.6421	51.6421	50.1421	47.6421	38.6421	0

Response EPT Richness

Year Sampled[Mining Status]

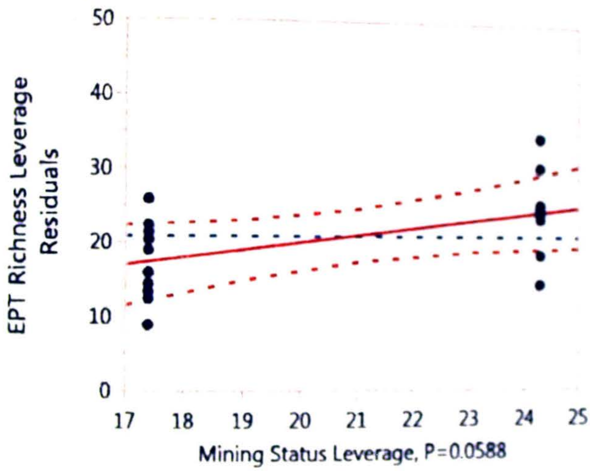
LSMeans Differences Tukey HSD

Level			Least Sq Mean
[un-mined]2011	A		39.000000
[un-mined]2008	A	B	29.000000
[mined]2008	A	B	28.500000
[mined]2011	A	B	26.000000
[un-mined]1981	A	B	20.000000
[un-mined]1980	A	B	17.500000
[un-mined]1979	A	B	16.000000
[mined]1979	A	B	14.500000
[mined]1980		B	9.000000
[mined]1981		B	9.000000

Levels not connected by same letter are significantly different.

Mining Status

Leverage Plot



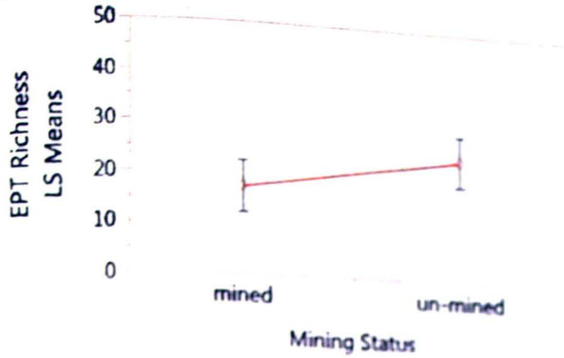
Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
mined	17.400000	2.2880122	17.4000
un-mined	24.300000	2.2880122	24.3000

Response EPT Richness

Mining Status

LS Means Plot

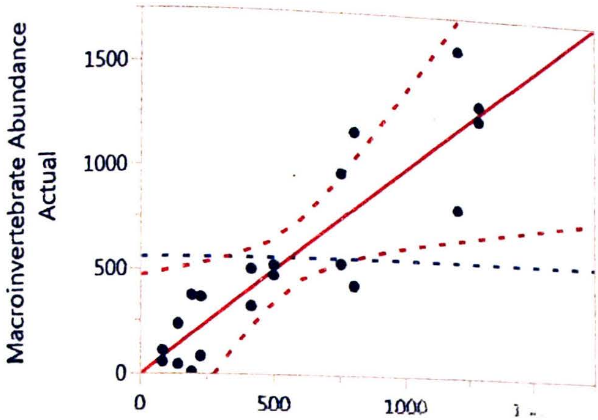


MACROINVERTEBRATE ABUNDANCE ANALYSIS: MINED VS. UN-MINED STREAMS

Response Macroinvertebrate Abundance

Whole Model

Actual by Predicted Plot



Summary of Fit

RSquare	0.808355
RSquare Adj	0.635874
Root Mean Square Error	285.0959
Mean of Response	559.75
Observations (or Sum Wgts)	20

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	3428355.3	380928	4.6866
Error	10	812796.5	81280	Prob > F
C. Total	19	4241151.8		0.0121*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	559.75	63.74937	8.78	<.0001*
Mining Status[mined]	-119.55	63.74937	-1.88	0.0902
Mining Status[mined]:Year Sampled[1979]	-299.2	180.3105	-1.66	0.1280
Mining Status[mined]:Year Sampled[1980]	-248.7	180.3105	-1.38	0.1979
Mining Status[mined]:Year Sampled[1981]	-356.7	180.3105	-1.98	0.0761
Mining Status[mined]:Year Sampled[2008]	846.8	180.3105	4.70	0.0008*
Mining Status[un-mined]:Year Sampled[1979]	-454.3	180.3105	-2.52	0.0304*
Mining Status[un-mined]:Year Sampled[1980]	-265.8	180.3105	-1.47	0.1712
Mining Status[un-mined]:Year Sampled[1981]	73.2	180.3105	0.41	0.6933
Mining Status[un-mined]:Year Sampled[2008]	524.2	180.3105	2.91	0.0156*

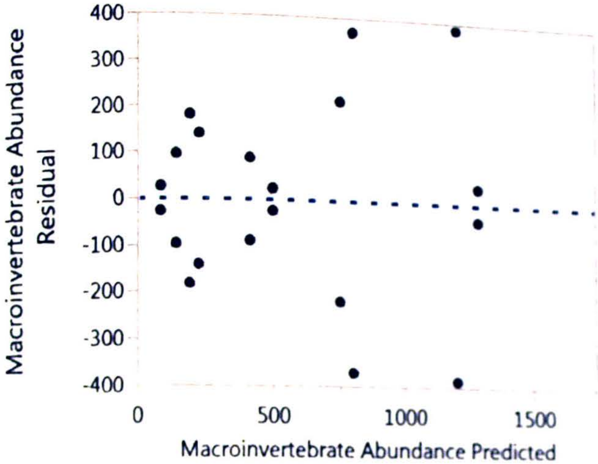
Response Macroinvertebrate Abundance

Whole Model

Effect Tests

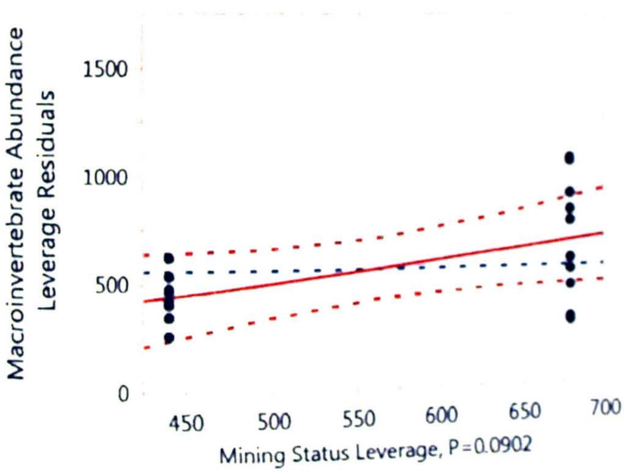
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Mining Status	1	1	285844.1	3.5168	0.0902
Year Sampled[Mining Status]	8	8	3142511.2	4.8329	0.0117*

Residual by Predicted Plot



Mining Status

Leverage Plot



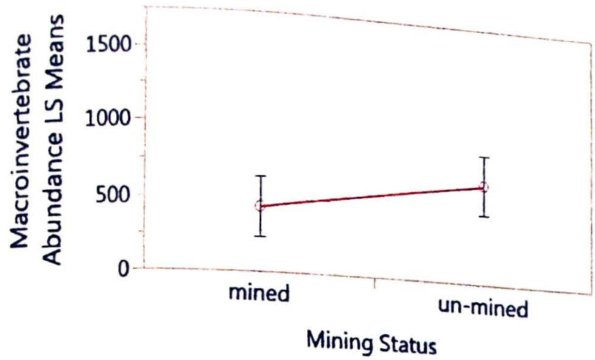
Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
mined	440.20000	90.155227	440.200
un-mined	679.30000	90.155227	679.300

Response Macroinvertebrate Abundance

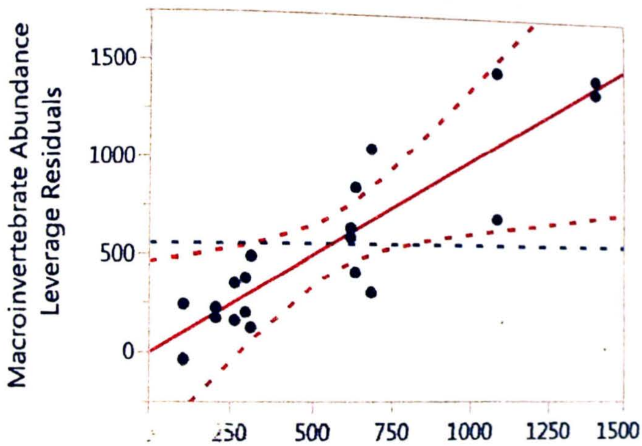
Mining Status

LS Means Plot



Year Sampled[Mining Status]

Leverage Plot



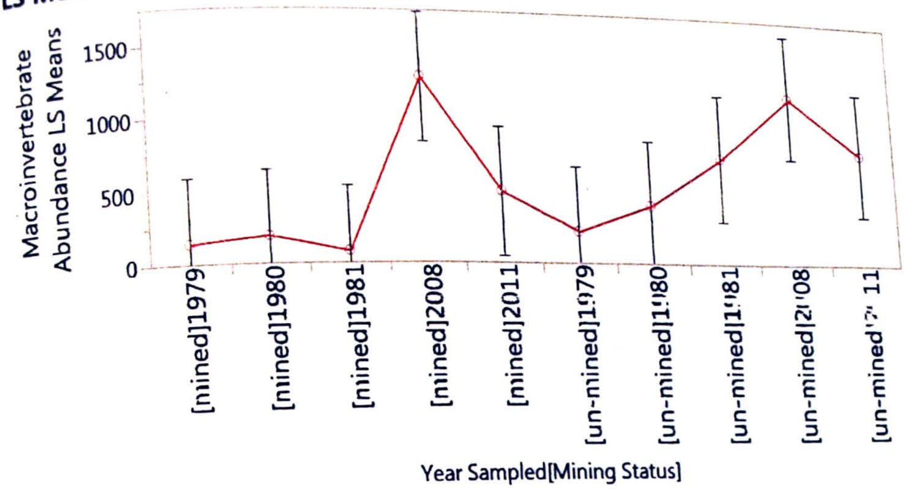
Least Squares Means Table

Level	Least Sq Mean	Std Error
[mined]1979	141.0000	201.59322
[mined]1980	191.5000	201.59322
[mined]1981	83.5000	201.59322
[mined]2008	1287.0000	201.59322
[mined]2011	498.0000	201.59322
[un-mined]1979	225.0000	201.59322
[un-mined]1980	413.5000	201.59322
[un-mined]1981	752.5000	201.59322
[un-mined]2008	1203.5000	201.59322
[un-mined]2011	802.0000	201.59322

Response Macroinvertebrate Abundance

Year Sampled[Mining Status]

LS Means Plot



LSMeans Differences Tukey HSD

α= 0.050 Q= 3.95864

Response Macroinvertebrate Abundance

Year Sampled[Mining Status]

LSMeans Differences Tukey HSD

LSMean[i]	Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	LSMean[j] [mined] 1979	[mined] 1980	[mined] 1981	[mined] 2008	[mined] 2011	[un-mined]1979	[un-mined]1980	[un-mined]1981	[un-mined]2008	[un-mined]2011
[mined]1979	Mean[i]-Mean[j]	0	-50.5	57.5	-1146	-357	-84	-272.5	-611.5	-1062.5	-661
	Std Err Dif	0	285.096	285.096	285.096	285.096	285.096	285.096	285.096	285.096	285.096
	Lower CL Dif	0	-1179.1	-1071.1	-2274.6	-1485.6	-1212.6	-1401.1	-1740.1	-2191.1	-1789.6
	Upper CL Dif	0	1078.09	1186.09	-17.408	771.592	1044.59	856.092	517.092	66.0919	467.592
[mined]1980	Mean[i]-Mean[j]	50.5	0	108	-1095.5	-306.5	-33.5	-222	-561	-1012	-610.5
	Std Err Dif	285.096	0	285.096	285.096	285.096	285.096	285.096	285.096	285.096	285.096
	Lower CL Dif	-1078.1	0	-1020.6	-2224.1	-1435.1	-1162.1	-1350.6	-1689.6	-2140.6	-1739.1
	Upper CL Dif	1179.09	0	1236.59	33.0919	822.092	1095.09	906.592	567.592	116.592	518.092
[mined]1981	Mean[i]-Mean[j]	-57.5	-108	0	-1203.5	-414.5	-141.5	-330	-669	-1120	-718.5
	Std Err Dif	285.096	285.096	0	285.096	285.096	285.096	285.096	285.096	285.096	285.096
	Lower CL Dif	-1186.1	-1236.6	0	-2332.1	-1543.1	-1270.1	-1458.6	-1797.6	-2248.6	-1847.1
	Upper CL Dif	1071.09	1020.59	0	-74.908	714.092	987.092	798.592	459.592	8.59188	410.092
[mined]2008	Mean[i]-Mean[j]	1146	1095.5	1203.5	0	789	1062	873.5	534.5	83.5	485
	Std Err Dif	285.096	285.096	285.096	0	285.096	285.096	285.096	285.096	285.096	285.096
	Lower CL Dif	17.4081	-33.092	74.9081	0	-339.59	-66.592	-255.09	-594.09	-1045.1	-643.59
	Upper CL Dif	2274.59	2224.09	2332.09	0	1917.59	2190.59	2002.09	1663.09	1212.09	1613.59
[mined]2011	Mean[i]-Mean[j]	357	306.5	414.5	-789	0	273	84.5	-254.5	-705.5	-304
	Std Err Dif	285.096	285.096	285.096	285.096	0	285.096	285.096	285.096	285.096	285.096
	Lower CL Dif	-771.59	-822.09	-714.09	-1917.6	0	-855.59	-1044.1	-1383.1	-1834.1	-1432.6
	Upper CL Dif	1485.59	1435.09	1543.09	339.592	0	1401.59	1213.09	874.092	423.092	824.592
[un-mined]1979	Mean[i]-Mean[j]	84	33.5	141.5	-1062	-273	0	-188.5	-527.5	-978.5	-577
	Std Err Dif	285.096	285.096	285.096	285.096	285.096	0	285.096	285.096	285.096	285.096
	Lower CL Dif	-1044.6	-1095.1	-987.09	-2190.6	-1401.6	0	-1317.1	-1656.1	-2107.1	-1705.6
	Upper CL Dif	1212.59	1162.09	1270.09	66.5919	855.592	0	940.092	601.092	150.092	551.592
[un-mined]1980	Mean[i]-Mean[j]	272.5	222	330	-873.5	-84.5	188.5	0	-339	-790	-388.5
	Std Err Dif	285.096	285.096	285.096	285.096	285.096	285.096	0	285.096	285.096	285.096
	Lower CL Dif	-856.09	-906.59	-798.59	-2002.1	-1213.1	-940.09	0	-1467.6	-1918.6	-1517.1
	Upper CL Dif	1401.09	1350.59	1458.59	255.092	1044.09	1317.09	0	789.592	338.592	740.092
[un-mined]1981	Mean[i]-Mean[j]	611.5	561	669	-534.5	254.5	527.5	339	0	-451	-49.5
	Std Err Dif	285.096	285.096	285.096	285.096	285.096	285.096	285.096	0	285.096	285.096
	Lower CL Dif	-517.09	-567.59	-459.59	-1663.1	-874.09	-601.09	-789.59	0	-1579.6	-1178.1
	Upper CL Dif	1740.09	1689.59	1797.59	594.092	1383.09	1656.09	1467.59	0	677.592	1079.09
[un-mined]2008	Mean[i]-Mean[j]	1062.5	1012	1120	-83.5	705.5	978.5	790	451	0	401.5
	Std Err Dif	285.096	285.096	285.096	285.096	285.096	285.096	285.096	285.096	0	285.096
	Lower CL Dif	-66.092	-116.59	-8.5919	-1212.1	-423.09	-150.09	-338.59	-677.59	0	-727.09
	Upper CL Dif	2191.09	2140.59	2248.59	1045.09	1834.09	2107.09	1918.59	1579.59	0	1530.09
[un-mined]2011	Mean[i]-Mean[j]	661	610.5	718.5	-485	304	577	388.5	49.5	-401.5	0
	Std Err Dif	285.096	285.096	285.096	285.096	285.096	285.096	285.096	285.096	285.096	0
	Lower CL Dif	-467.59	-518.09	-410.09	-1613.6	-824.59	-551.59	-740.09	-1079.1	-1530.1	0
	Upper CL Dif	1789.59	1739.09	1847.09	643.592	1432.59	1705.59	1517.09	1178.09	727.092	0

Response Macroinvertebrate Abundance

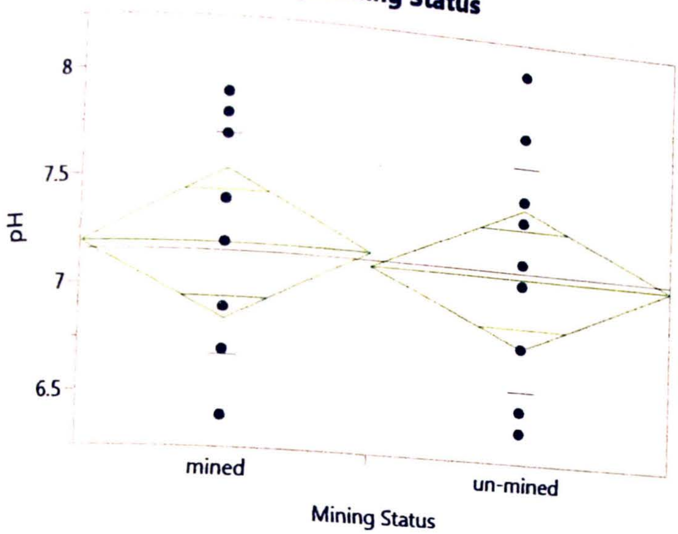
Year Sampled[Mining Status]

LSMeans Differences Tukey HSD

Level			Least Sq Mean
[mined]2008	A		1287.0000
[un-mined]2008	A	B	1203.5000
[un-mined]2011	A	B	802.0000
[un-mined]1981	A	B	752.5000
[mined]2011	A	B	498.0000
[un-mined]1980	A	B	413.5000
[un-mined]1979	A	B	225.0000
[mined]1980	A	B	191.5000
[mined]1979		B	141.0000
[mined]1981		B	83.5000

Levels not connected by same letter are significantly different.

Oneway Analysis of pH By Mining Status



Oneway Anova

Summary of Fit

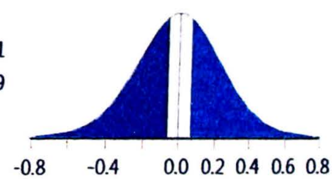
Rsquare	0.003925
Adj Rsquare	-0.0485
Root Mean Square Error	0.524696
Mean of Response	7.157143
Observations (or Sum Wgts)	21

t Test

un-mined-mined

Assuming equal variances

Difference	-0.06273	t Ratio	-0.27361
Std Err Dif	0.22926	DF	19
Upper CL Dif	0.41711	Prob > |t|	0.7873
Lower CL Dif	-0.54257	Prob > t	0.6063
Confidence	0.95	Prob < t	0.3937



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Mining Status	1	0.0206104	0.020610	0.0749	0.7873
Error	19	5.2308182	0.275306		
C. Total	20	5.2514286			

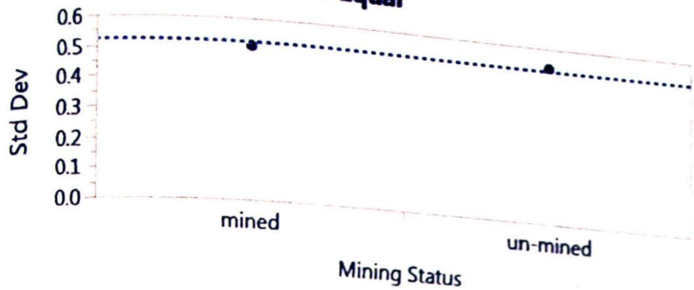
Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
mined	10	7.19000	0.16592	6.8427	7.5373
un-mined	11	7.12727	0.15820	6.7962	7.4584

Std Error uses a pooled estimate of error variance

Oneway Analysis of pH By Mining Status

Tests that the Variances are Equal



Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
mined	10	0.5130519	0.4120000	0.4100000
un-mined	11	0.5349596	0.4297521	0.4272727

Test	F Ratio	DFNum	DFDen	p-Value
O'Brien[.5]	0.0305	1	19	0.8632
Brown-Forsythe	0.0191	1	19	0.8914
Levene	0.0209	1	19	0.8866
Bartlett	0.0157	1	.	0.9003
F Test 2-sided	1.0872	10	9	0.9095

Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
0.0752	1	18.935	0.7869

t Test
0.2742

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
mined	10	113.500	110.000	11.3500	0.212
un-mined	11	117.500	121.000	10.6818	-0.212

2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
113.5	0.21208	0.8320

1-way Test, ChiSquare Approximation

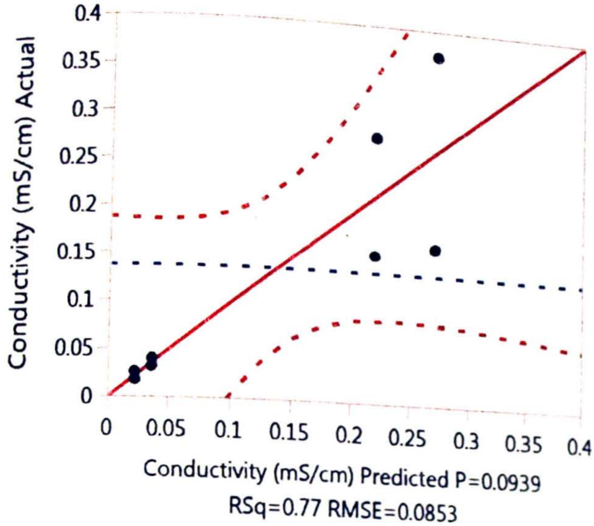
ChiSquare	DF	Prob>ChiSq
0.0612	1	0.8046

CONDUCTIVITY ANALYSIS: MINED VS. UN-MINED STREAMS

Response Conductivity (mS/cm)

Whole Model

Actual by Predicted Plot



Summary of Fit

RSquare	0.766512
RSquare Adj	0.591395
Root Mean Square Error	0.085286
Mean of Response	0.137625
Observations (or Sum Wgts)	8

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.09551338	0.031838	4.3772
Error	4	0.02909450	0.007274	**Prob > F**
C. Total	7	0.12460788		0.0939

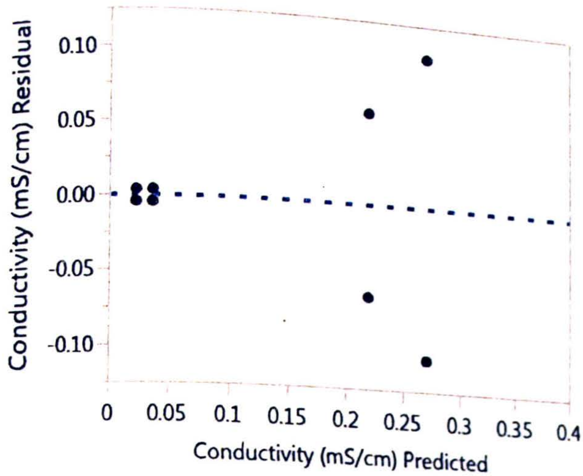
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.137625	0.030153	4.56	0.0103*
Year[2008]	0.016375	0.030153	0.54	0.6159
Mining Status[mined]	0.107625	0.030153	3.57	0.0234*
Year[2008]*Mining Status[mined]	0.009375	0.030153	0.31	0.7714

Response Conductivity (mS/cm)

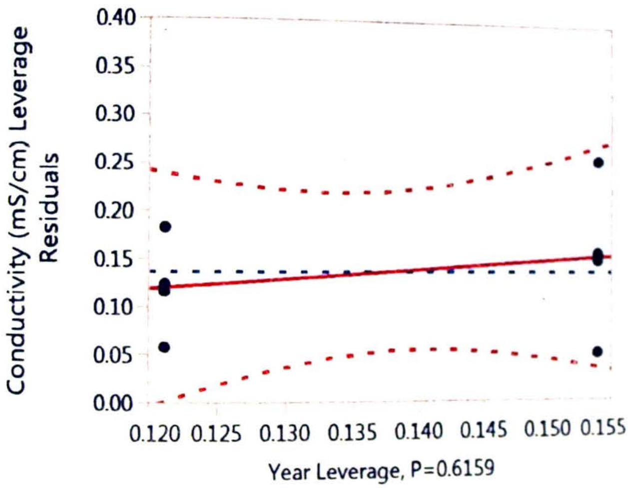
Whole Model

Residual by Predicted Plot



Year

Leverage Plot



Least Squares Means Table

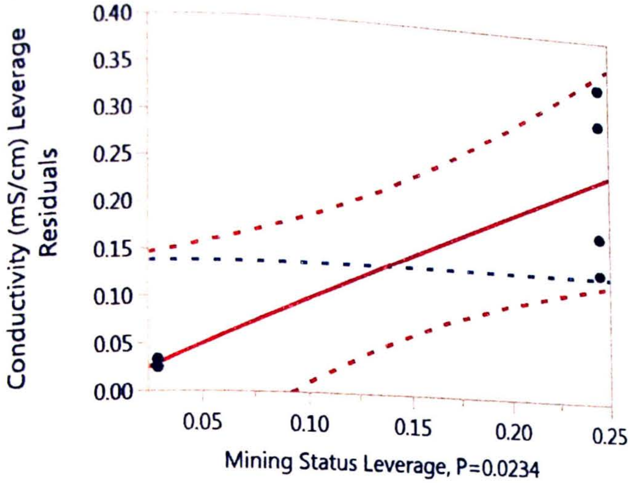
Level	Least Sq Mean	Std Error	Mean
2008	0.15400000	0.04264277	0.154000
2011	0.12125000	0.04264277	0.121250

Mining Status

Response Conductivity (mS/cm)

Mining Status

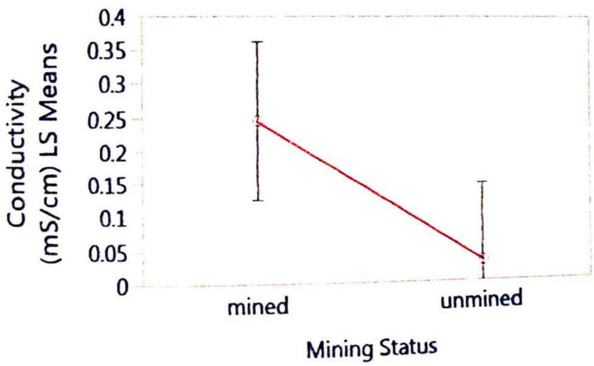
Leverage Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
mined	0.24525000	0.04264277	0.245250
unmined	0.03000000	0.04264277	0.030000

LS Means Plot

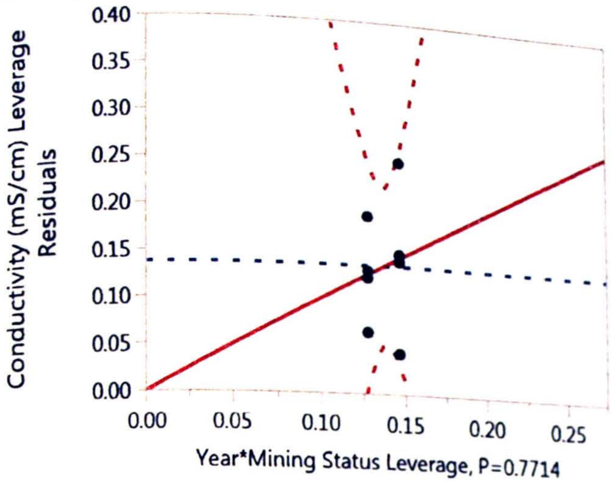


Year*Mining Status

Response Conductivity (mS/cm)

Year*Mining Status

Leverage Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error
2008,mined	0.27100000	0.06030599
2008,unmined	0.03700000	0.06030599
2011,mined	0.21950000	0.06030599
2011,unmined	0.02300000	0.06030599

APPENDIX D: MISCELLANEOUS TABLES

Table 1. A Brief Description of Previous Studies Conducted in Study Area.

Authors	Streams Studied	Brief Summary
Minear and Tschantz, 1976	Bowling Branch, Bill's Branch, Green Branch, Indian Fork, and Lowe Branch.	Analysis of chemical and mineral content in water.
Vaughan et al., 1978	24 streams including: Sugar Camp Creek, Louse Creek, Bowling Branch, Duncan Branch, Ursery Creek, Green Branch, Indian Fork, and Lowe Branch	Surveyed aquatic insect, fish, and diatom diversity.
Vaughan, 1979	Bill's Branch, Green Branch, Indian Fork, and Lowe Branch.	Analysis of fish and diatom diversity.
Tolbert and Vaughan, 1980	Bill's Branch, Green Branch, Indian Fork, and Lowe Branch	Surveyed Aquatic insect diversity and abundance
Schiller, 1986	Bruce Hallow and Crabapple	Aquatic insect diversity, POM[a], and secondary productivity.
Dickens et al, 1989	Bowling Branch, Bill's Branch, Ursery Branch, Green Branch, Indian Fork, and Lowe Branch.	Survey of chemical and mineral fluctuations.

Table 2. Tennessee Macroinvertebrate Index (TDEC, 2006)

Metric	Definition
EPT (Ephemeroptera, Plecoptera, Trichoptera) Richness	Sum of Ephemeroptera, Plecoptera, and Trichoptera taxa.
TR (Taxa Richness)	Sum of all taxa
%OC (Percent oligochaetes and chironomids)	%OC = {(total number of Oligochaeta + Chironomidae)/(total number of individuals in the subsample)}X100
%EPT (EPT Abundance)	%EPT = {(Sum of Ephemeroptera, Plecoptera, Trichoptera count)/ (total number of individuals in the subsample)}X100
NCBI (North Carolina Biotic Index)	NCBI = Sum of $x_i t_i /n$ x_i = number of individuals within a taxon t_i = tolerance value of a taxon n = total number of individuals in the subsample.
%NUTOL (Percent Nutrient tolerant organisms)	%NUTOL = {(Total number of *Chuematopsyche, Lirceus, Physella, Baetis, Psephenus, Stenelmis, Simulium, Elimia, Oligochaeta, Polypedilum, Rheotanytarsus, Stenacron, Cricotopus, and Chironomus*)/ (total individuals in the sample)} X100
%Clingers (Percent contribution of organisms that build fixed retreats or have adaptations to attach to surfaces in flowing water)	%Clingers = {(total of clinger individuals)/(total individuals in the sample)}X100

Table 3. History of Mining in Study Stream Watersheds (Whitley, 2009).

Stream	Mining History	Reference
Bruce Creek	approximately 1965	Schiller (1986).
Green Branch	1972-75 and 2007	Schiller (1986), Minear and Tschantz. (1976), and Dickens et al. (1989.
Bowling Branch	1976-1978	Minear and Tschantz. (1976), and Dickens et al. (1989).
Bill's Branch	1974-75 and 2004	Dickens et al. (1989), Minear and Tschantz (1976).
Crabapple Creek	No mining	Schiller (1986).
Lowe Branch	No mining	Schiller (1986), Dickens et al. (1989).

Table 4. **Habitat assessments for six streams studied in the Big South Fork Watershed in East Tennessee.**

Stream	Habitat Assessment	Date
Bruce Creek	Not Impaired	6/18/2008
Bills Branch	Moderately Impaired	6/19/2008
Bowling Branch	Moderately Impaired	7/23/2008
Green Branch	Moderately Impaired	6/19/2008
Lowe Branch	Not impaired	7/22/2008
Crabapple Creek	Not Impaired	7/23/2008

Table 5. pH levels in mined vs. un-mined streams from 1979, 1980, 1981, 2008 and 2011.

Mined Streams		Un-Mined Streams		
Green	Bill's	Crabapple	Lowe	Reference (Year Sampled)
6.7	6.4	7.2	6.5	Vaughan et al., 1982 (1979)
7.9	7.8	8.1	7.8	Vaughan et al., 1982 (1980)
7.7	7.2	7.5	7.4	Vaughan et al., 1982 (1981)
7.4	7.2	6.8	6.8	Whitley, 2009 (2008)
6.7	6.9	7.1	6.8	Current study (2011)

Table 6. Conductivity in mined vs. un-mined streams from 2008 and 2011.

Mined Streams		Un-Mined Streams		
Green	Bill's	Crabapple	Lowe	Study (Year Sampled)
0.374	0.168	0.033	0.041	Whitley, 2009 (2008)
0.282	0.157	0.019	0.027	Present Study (2011)

Table 7. Taxa richness scores of 4 pairs of composited Surber samples collected from mined vs. un-mined streams in 1979, 1980, 1981, 2008 and 2011

Mined Streams		Un-Mined Streams		
Green	Bill's	Lowe	Crabapple	Study (Year Sampled)
7	30	26	13	Vaughan et al., 1982 (1979)
4	16	24	23	Vaughan et al., 1982 (1980)
6	13	21	31	Vaughan et al., 1982 (1981)
47	44	54	53	Whitley, 2009 (2008)
38	32	39	70	Present Study (2011)

Table 8. EPT richness of four pairs of composited Surber samples collected from mined vs. un-mined streams in 1979, 1980, 1981, 2008 and 2011.

Mined Streams		Un-Mined Streams		
Green	Bill's	Lowe	Crabapple	Study (Year Sampled)
6	23	22	10	Vaughan et al., 1982 (1979)
4	14	18	17	Vaughan et al., 1982 (1980)
6	12	20	20	Vaughan et al., 1982 (1981)
30	27	30	28	Whitley, 2009 (2008)
30	22	29	49	Present Study (2011)

Table 9. Multimetric scores of four pairs of composited Surber samples collected from mined vs. un-mined streams in 1979, 1980, 1981, 2008 and 2011.

Mined Streams		Un-Mined Streams		
Green	Bills	Lowe	Crabapple	Study (Year Sampled)
18	40	40	34	Vaughan et al., 1982 (1979)
30	36	40	40	Vaughan et al., 1982 (1980)
32	36	40	40	Vaughan et al., 1982 (1981)
38	36	28	38	Whitley, 2009 (2008)
42	42	42	38	Present Study (2011)

Table 10. Total number of macroinvertebrates collected in four pairs of composited Surber samples collected from mined vs. un-mined streams in 1979, 1980, 1981, 2008, 2011.

Mined		Un-mined		
Green	Bill's	Lowe	Crabapple	Study (Year Sampled)
45	237	366	84	Vaughan et al., 1982 (1979)
9	374	502	325	Vaughan et al., 1982 (1980)
57	110	970	535	Vaughan et al., 1982 (1981)
1323	1251	817	1590	Whitley, 2009 (2008)
522	474	432	1172	Present Study (2011)

Table 11. **Comparison of bioassessments using means of bioassessments on each of eight paired Surber samples, Whitley (2009) versus bioassessments of four composited pairs of Surber samples *kicknet samples in Whitley's study.**

Stream	Mining Status	2008 Bioassessment	2011 Bioassessment
Bills Branch	Mined	Not Impaired – 6/19/08	Not Impaired – 4/10/11
Green Branch	Mined	Not Impaired – 6/19/08	Not Impaired – 4/10/11
Bruce Creek	Mined	Slightly Impaired – 6/18/08*	Not Impaired – 4/09/11
Bruce Creek	Mined	Slightly Impaired – 7/23/08*	N/A
Bowling Branch	Mined	Slightly Impaired – 6/19/08*	Not Impaired – 4/10/11
Bowling Branch	Mined	Not Impaired – 7/23/08*	N/A
Lowe Branch	Un-Mined	Slightly Impaired – 7/22/08	Not Impaired – 4/09/11
Crabapple Creek	Un-Mined	Not Impaired – 7/23/08	Not Impaired – 4/09/11

Table 12. Taxa richness of four pairs of composited Surber samples collected from Crabapple Creek in 1979, 1980, 1981, 2008 and 2011. Crabapple is an un-mined, "pristine" ecoregion (69D04) reference stream.

Crabapple	Study (Year Sampled)
13	Vaughan et al., 1982 (1979)
23	Vaughan et al., 1982 (1980)
31	Vaughan et al., 1982 (1981)
53	Whitley, A., 2009 (2008)
70	Present Study (2011)

Table 13. EPT richness in Crabapple Creek from 1979, 1980, 1981, 2008 and 2011. Crabapple Creek is an un-mined, "pristine" ecoregion (69D04) reference stream.

Crabapple	Study (Year Sampled)
10	Vaughan et al., 1982 (1979)
17	Vaughan et al., 1982 (1980)
20	Vaughan et al., 1982 (1981)
28	Whitley, A., 2009 (2008)
49	Present Study (2011)

Table 14. Multimetric scores for Crabapple Creek from 1979, 1980, 1981, 2008 and 2011. Crabapple is an un-mined, "pristine" ecoregion (69D04) reference stream.

Multimetric score	Study (Year Sampled)
34	Vaughan et al. 1982 (1979)
40	Vaughan et al. 1982 (1980)
40	Vaughan et al. 1982 (1981)
36	Whitley, A., 2009 (2008)
38	Present Study (2011)

Table 15. Comparison of Multimetric Score means vs. means of composited data.

Stream	Composited Mean - 2008	Multimetric Score Means - 2011
Green Branch	39	25
Bill's Branch	33	33
Crabapple Creek	33	32
Lowe Branch	26	31