Kentucky Mountaintop Mining Benthic Macroinvertebrate Survey

Central Appalachian Ecoregion, Kentucky

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EXECUTIVE SUMMARY

In response to a request by the EPA Region 4 Water Management Division, an assessment of stream macroinvertebrate community health was conducted by EPA Region 4 Science and Ecosystem Support Division staff at 12 sites in the Eastern Coalfield area of Kentucky, May 1-4, 2000. The study was designed to determine if streams in mined watersheds were being impacted by a practice known as "mountaintop mining and valley fill " (MTM/VF). This mining approach consists of disrupting or removing the tops of mountains to access multiple coal seams, and depositing the bulk of the overburden in adjacent valleys burying first- and second-order streams under tons of soil and rock.

The eight mining-related sites selected for this study were located in Breathitt, Perry, Knot, and Bell Counties. These locations represent sites downstream of active mining, inactive mining and/or reclaimed mining sites. Four reference sites were located in the Robinson Forest and Redbird Wildlife Management Areas located in Breathitt, Knott, Clay, and Leslie Counties, areas within which mining has not occurred. At each study site, a habitat evaluation was performed, *in situ* water quality was measured, and macroinvertebrate samples were collected. In addition, sediment characterization samples were collected at eight of the 12 sites. Habitat evaluation, collection of macroinvertebrates, and interpretation of results were based on US EPA Rapid Bioassessment Protocols and EPA Region 4 Standard Operating Procedures. Sediment characterization sampling and interpretation techniques followed US EPA EMAP protocols.

Various measures of *in situ* water quality, habitat quality and macroinvertebrate community structure were found to be related to mining activities. In particular, conductivity was considerably higher at all mined sites than it was at reference sites. Conductivity showed the strongest correlation to indicators of macroinvertebrate community health (i.e., % ephemeroptera, taxa richness, EPT index, biotic index, and MBI) suggesting this as either a route by which impairment occurred in mined areas, or that conductivity is a surrogate for other factors that were not measured. Severe impact to the mayfly (Ephemeroptera) fauna was exhibited at all mined sites. Habitat scores, generally lower at sampling locations downstream of mined areas than at reference sites, were correlated to several measures of diversity and dominance of key groups of macroinvertebrates. Especially noted was the decrease in pollution-sensitive macroinvertebrates (Ephemeroptera, Plecoptera, and Trichoptera) at the mined watersheds. Sediment deposition scores were also strongly correlated with conductivity.

In summary, impacts of MTM/VF activities in eastern Kentucky were evident based on stream biological and habitat indicators. Mine sites generally had higher conductivity, greater sediment deposition, smaller substrate particle sizes, and a decrease in pollution sensitive macroinvertebrates with an associated decrease in taxa diversity compared to reference sites.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

APPENDICES

1.0 INTRODUCTION

The purpose of this study was to evaluate the ecological health of first to third order streams subjected to mountain top mining/valley fill (MTM/VF) practices in the Central Appalachian Ecoregion of Kentucky (Omernik 1987). Mountaintop mining is the term that describes a mining practice in which millions of tons of dirt and rock are removed from mountaintops in order to extract multiple seams of coal. The resulting overburden is often placed in the adjacent valleys resulting in the stream being completely filled or receiving excessive sedimentation. Both pre-mining deforestation and mountaintop mining lead to accelerated sediment deposition, disrupted hydrology, and habitat degradation affecting the stream biota. The U.S. Fish and Wildlife Service (1998), in an inventory of Kentucky mining permits issued pursuant to the Surface Mining Control and Reclamation Act report that the Department for Surface Mining Reclamation authorized impacts to 354 miles of streams during the permitting period of April 1986 through July 1995. This included the authorization of placement of overburden in 180 miles of streams, and impacts to an additional 152 miles of streams between valley fills and the downstream sediment retention structures.

2.0 BACKGROUND

EPA Region 4 staff participated in meetings with EPA Region 3, U.S. Fish and Wildlife Service, U.S. Office of Surface Mining, West Virginia Department of Environmental Protection, the Kentucky Division of Water, the Kentucky Department of Fish and Wildlife Resources, and the U.S. Army Corps of Engineers to discuss the environmental impacts associated with mountaintop mining operations. These agencies are currently collaborating to develop an Environmental Impact Statement (EIS) relative to mountaintop mining practices in the Central Appalachian Ecoregion.

In response to ecological concerns and a lack of available information, the EPA Region 4, Water Management Division requested that the EPA Region 4 Science and Ecosystem Support Division evaluate the ecological health of streams associated with mountaintop mining activities.

3.0 STUDY AREA

The study area is located in the Central Appalachian Ecoregion of eastern Kentucky. This area, referred to as the Eastern Coalfield, contains rich deposits of bituminous coal. Stretching from the Appalachian Mountains westward across the Cumberland Plateau, the Eastern Coalfield encompasses much of eastern Kentucky. The Central Appalachian Ecoregion is primarily a rugged plateau composed of sandstone, shale, conglomerate, and coal vegetated by a mixed mesophytic forest. The rugged terrain, cool climate, and infertile soils limit agriculture in this region.

Using land use and cover type information on permitted mining sites, Kentucky orthoquad maps, and information from the Kentucky Division of Water (KDOW), watersheds were selected in areas of active mountaintop mining or recently closed mines. Eight study watersheds were selected, ranging in size from approximately 2 to16 square miles (Figure 1, Table 1). Attempts were made to avoid locating study watersheds in the vicinity of residential areas or permitted municipal/industrial (non-mining) discharges. As a result, only one station (Lost Creek, Station 9) had possible influences from straight pipes (direct discharges of untreated sewage from private residences) and a permitted figure 1

discharge. In addition, a permitted discharge (Perry County school) was located on Sixteen Mile Creek, a tributary to Lost Creek approximately 4.2 miles upstream of Station 9. All stream stations in mined areas were located downstream of the sediment retention ponds that were constructed as part of the mining process. The selected watersheds were classified in the following categories relative to mountaintop mining operations: inactive (old mining), active/inactive, active/reclaimed, and unmined (forested reference) watersheds (Table 1).

Table 1. Stream sampling locations, Eastern Kentucky. May 1-4, 2000.

Stream	Station	Locale	Latitude/ Longitude	County	Drainage area (sq. mi)	Mining Status
Long Fork	1	Buckhorn Cr. Road	37 26.78461 83 11.2066	Breathitt	8.105	active / inactive
Buffalo Creek	3	Fourseam Road	37 13.5054 83 10.3722	Perry	2.755	inactive
Laurel Fork	4	Upper Laurel Fork Road	37 26.4033 83 12.46167	Breathitt/ Perry	3.735	active / inactive
Fugate Branch	5	Fugate Fork Road	37 27.55833 83 14.22333	Breathitt	2.661	active / inactive
Sims Fork	6	Sims Fork Road	36 50.51167 83 36.38667	Bell	6.323	active / reclaimed
Spring Fork/ Quicksand Creek	7	near confluence with Hughes Creek	37 32.905 83 03.815	Breathitt	12.007	active / inactive
Lost Creek	9	SR 1446	37 23.78 83 16.013	Perry	16.858	active / inactive
Lick Branch	14	Cyprus AMAX WMA	37 23.275 83 08.31	Knott/ Perry	3.212	active / inactive
Clemons Fork (Ref)	10	Robinson Forest	37 27.97667 83 09.12833	Breathitt	5.016	unmined
Coles Fork (Ref)	11	Robinson Forest, Buckhorn Ck. Road	37 27.8522 83 07.81434	Knott/ Breathitt	6.115	unmined
Big Double Cr. (Ref)	12	FR 1501	37 06.050 83 35.51	Clay	3.716	unmined
Sugar Cr. (Ref)	13	Redbird WMA	37 07.576 83 32.446	Leslie/ Clay	4.421	unmined

Ref - reference stream

Four reference watersheds were selected in the Robinson Forest and the Redbird WMA (Table 1, Figure 1). Reference watersheds were selected based on the absence of mining activity, proximity to test sites, similar stream order, and recommendations by the KDOW.

4.0 STUDY METHODS

Rapid Bioassessment Protocol III (RBP III) developed by EPA (Plafkin et al. 1989, Barbour et al. 1999) was used to evaluate impacts to these streams. Included in the RBP III are measures of *in situ* water quality and evaluations of the physical habitat which indicate the streams' chemical and physical status. The benthic macroinvertebrate community is the indicator of biological condition. Substrate size, one of the most important determinants of habitat for fish and macroinvertebrates in streams, was determined using EPA Environmental Monitoring and Assessment Program (EMAP) protocols (Kaufmann and Robison 1998, Kaufmann et al. 1999). The substrate characterization was used to evaluate differences in stream bed composition between reference and test sites. Study methods are described below.

4.1 *In Situ* Water Quality

In situ water quality measurements included instantaneous measurements of pH, conductivity, water temperature, and dissolved oxygen. These measurements serve to identify water quality conditions which may affect aquatic life. *In situ* water quality measurements were made prior to collection of macroinvertebrates and habitat evaluations. Hydrolab® multi-parameter field instruments, calibrated prior to daily use, were positioned at approximately 0.5 feet in the water column in an undisturbed area of the study station. All *in situ* water quality measurements were recorded in the field log along with appropriate station information (station number, date, time).

At the end of each sampling day, field instruments used to measure *in situ* water quality were checked for calibration. Results of both pre- and post- sampling instrument calibration were recorded in the field log.

4.2 Macroinvertebrate Sample Collection

Methods used in this study (Plafkin et al. 1989, Barbour et al. 1999, U.S. EPA 2000a) evaluate the status of the benthic macroinvertebrate community. Due to their limited mobility and relatively long life span, benthic macroinvertebrates integrate and reflect water quality effects over time and are excellent indicators of stress in aquatic systems. Rapid Bioassessment Protocol III (RBP III) requires the most intense level of effort of the protocols, followed by identifying macroinvertebrates to at least genus level. Benthic macroinvertebrates were collected from multiple habitats as follows:

> riffles - 3 "kicks" in the faster current and 3 "kicks" in the slower current with a standard D-frame biological dip net (800 X 900 μm mesh),
> snags/woody debris - 5-6 pieces (~1' length) washed in sieve bucket or standard D-frame biological dip net,
> leaf packs - equivalent to one half dip net,
> undercut banks - 6 "jabs" with standard D-frame biological dip net, and
> bottom substrate - 3 sweeps (disturb sediment to 3 cm depth).

Benthic macroinvertebrate samples were stored in plastic, one quart containers in ethanol (90%). Sample containers were labeled both inside and outside with labels containing the following information: station number, stream name, date and time of collection, and sample type. Samples were checked for adequate preservation at the end of the daily sampling and secured in locked field vehicles until returned to the laboratory where they were sorted under lighted magnification, and then identified and enumerated with the aid of microscopy.

Staff of the KDOW have developed collection methods, tentative scoring criteria for core metrics, and a tentative scoring index referred to as the Macroinvertebrate Bioassessment Index (MBI), for small, headwater streams (1st - 2nd order) in eastern Kentucky (Pond and McMurray 2000). These scoring criteria were developed based on sampling of 42 sites (25 reference and 17 test) scattered throughout the Central and Southwestern Appalachians of Kentucky. Reference streams were located in highly forested, undisturbed areas, whereas test sites ranged from slightly to severely impacted by mining, logging, and residential development. The core metrics used in this index represent four major measures of benthic community health:

1) Richness -- Taxa Richness, EPT Index
2) Composition -- %Ephemeroptera, %Chironomidae + Oligochaeta
3) Tolerance -- Biotic Index, and
4) Habit -- % Clingers.

In discussions prior to this study, biologists from KDOW and EPA Region 4 determined that sampling methods utilized by both agencies were similar both in extent and the approach used to select habitats to be sampled. In order to provide data that are consistent with and complimentary to those of KDOW, riffle kick samples were kept separate from the composite sample for other habitats during sampling and identification. KDOW uses this approach to evaluate the relationship between sediment and biota in productive riffle habitats. This differs from the RBP III protocol and usual EPA Region 4 sampling methods. For data evaluation, the percent metrics (Ephemeroptera, Clingers, Chironomidae + Oligochaeta) and biotic index were calculated from riffle samples only, while taxa richness and EPT index were calculated from both riffle and multihabitat samples combined.

4.3 Habitat Evaluation

Physical habitat quality is a major determinant of biological diversity of stream benthic macroinvertebrate communities. Habitat evaluation results, when compared to reference sites, identify degraded conditions and the severity of such degradation. The High Gradient Habitat Evaluation Form (Barbour et al. 1999) was utilized during this study. Parameters assessed as part of the habitat evaluation include epifaunal substrate, embeddedness, velocity/depth regime, sediment deposition, channel characteristics, bank stability, vegetative cover, and riparian zone integrity.

4.4 Substrate Characterization

Substrate characteristics are important determinants of habitat for fish and macroinvertebrates in streams (Kaufmann and Robison 1998, Kaufmann et al. 1999), and are often sensitive indicators of anthropogenic impacts on streams (Minshall et al 1985). Substrate size characterization was used to evaluate reference versus test sites. Cobble-sized substrate provides the greatest amount of usable habitat to benthic macroinvertebrates, while smaller sized substrate offers reduced habitat for colonization (Green et al. 2000).

Substrate size characterization was performed using EMAP protocols (Kaufmann and Robison 1998, Kaufman et al. 1999). Eleven transects were assessed in each 100 meter reach. The middle transect was located in the riffle where the biological sample was collected. Five transects were located upstream of the middle transect and five downstream of the middle transect. Transects were spaced at 10 meter intervals. Five substrate particles (e.g., cobble, sand, gravel, etc.) selected at evenly spaced intervals across each transect (left, left middle, middle, right middle, and right) were measured (to the nearest millimeter), recorded, and classified. A total of 55 particle measurements were made at each station.

Particle measurements were used to determine the proportion of bedrock, boulder, cobble, coarse gravel, fine gravel, and sand and fines present in the reach, according to Wentworth size classes as described in Wolman (1954). Particles with diameter less than 2 mm were differentiated into specific sand-sized fractions (e.g., 0.125, 0.250, 0.500, 1.00 mm) with the aid of a waterproof "sand card" or identified as silt/clay (<0.062 mm) (Pruitt et al. 1999). The 55 particle measurements were also used to determine the mean particle size in the reach. Since the transects were evenly spaced, the riffle and pool habitat within the reach was sampled in proportion to their presence in the reach. For example, if the 100 meter reach was 20% pool and 80% riffle, then the measurements generally occurred 20% of the time in the pools and 80% of the time in riffles. Bankfull depth, thalweg (the location of the deepest part of the channel), slope, and wetted width were also recorded for each transect. Bankfull depth was estimated by identifying field indicators of bankfull stage (e.g., the top of well-established point bars, vegetation, and/or lichen lines, etc.). Thalweg, slope, and wetted width were measured directly.

5.0 QUALITY ASSURANCE/QUALITY CONTROL

Field and laboratory methods utilized on this project followed EPA approved methodology (Plafkin et al. 1989, Barbour et al. 1999, U.S. EPA 2000a, U.S. EPA 2000b). To provide an indication of field and laboratory precision, duplicate macroinvertebrate samples were collected at two of the 12 sampling sites as determined by the field team leader.

Field instruments utilized during the *in situ* water quality studies were calibrated before and after daily field sampling according to manufacturer's instructions and U.S. EPA (2000b). Calibration results were recorded in the field log book and signed by the project investigator.

6.0 RESULTS AND DISCUSSION

6.1 *In Situ* Water Quality

In situ water quality measurements (pH, conductivity, water temperature, and dissolved oxygen) were collected at each of the 12 study sites (Table 2, Appendix A). The most noticeable *in situ* water quality parameter was elevated conductivity values observed at watersheds associated with MTM/VF operations. EPA Region 3 reported similar findings in recent studies of watersheds in West Virginia associated with mountaintop mining operations (Green et al. 2000). Conductivity values at the test sites ranged from 420 to 1690 µmhos/cm with an average of 994 µmhos/cm (Table 2). When compared to the range (29.9 - 65.8 µmhos/cm) and mean (46.75 µmhos/cm) at the reference watersheds, conductivity at the test sites was 21 times higher.

Table 2. *In situ* water quality measurements, Eastern Kentucky. May 1-4, 2000.

Station #	Stream	Date	Time	D.O. mg/L	Temp. °C	pH Units	Cond. µmhos/cm
1	Long Fork	05/02/00	1300	9.34	15.16	8.08	1310
3	Buffalo Creek	05/03/00	1500	8.44	18.18	8.01	784
4	Laurel Fork	05/03/00	0915	9.54	13.66	7.64	1550
5	Fugate Branch	05/02/00	1305	9.58	15.00	8.19	836
6	Sims Fork	05/03/00	1500	8.52	18.57	8.14	420
7	Spring Fk/Quicksand Cr.	05/02/00	1000	9.17	15.01	7.15	480
9	Lost Creek	05/02/00	1500	9.69	15.97	7.99	881
14	Lick Branch	05/04/00	1005	8.92	16.33	8.16	1690
10 REF	Clemons Fork	05/02/00	1500	9.50	15.40	7.08	65.8
11 REF	Coles Fork	05/02/00	1015	9.44	13.00	7.13	40.6
12 REF	Big Double Creek	05/03/00	1300	9.13	14.30	7.32	50.7
13 REF	Sugar Creek	05/03/00	1000	9.60	12.28	7.42	29.9

REF - reference watershed

The range of observed pH values (Table 2) at watersheds associated with mountaintop mining operations (7.15 to 8.19) was higher than that of the reference watersheds (7.08 to 7.42). This finding is consistent with that observed in EPA Region 3 studies where mined areas exhibited higher pH (Green et al. 2000). Only one test site, Station 7, had a pH that was within the range of pH values observed at the reference watersheds; all other test sites exceeded a pH of 7.6.

In situ water temperature was generally higher at the test sites than at the reference sites (Table 2; Appendix A). Water temperature measurements were made in the morning, midday, or afternoon. Three morning (9:00 - 10:00 a.m.) measurements of water temperature at the test sites ranged from 13.66 to 16.33 ºC, while reference sites were 12.28 and 13.00 ºC for the same period (Table 2).

Midday (1:00 p.m.) water temperatures at two test sites were 15.16 and 15.00 ºC, while midday water temperature measured at one of the reference sites was 14.30 ºC. Afternoon (3 p.m.) measurements of water temperature at three of the test sites ranged from 15.97 to 18.57 ºC while measurement of water temperature at a reference site during this same period was 15.40 ºC.

Dissolved oxygen values at the test sites ranged from 8.44 to 9.69 mg/L while reference sites ranged from 9.13 to 9.60 mg/L (Table 2). As illustrated by the box and whisker plot (Appendix A), dissolved oxygen values at the test sites exhibited a greater variation over the morning through afternoon period than was observed at the reference sites.

6.2 Benthic Macroinvertebrates

Benthic macroinvertebrates were identified to genus level (Appendix B). As discussed previously, the choice of core metrics was consistent with Kentucky's Macroinvertebrate Bioassessment Index (MBI) for Headwater Streams of the Eastern Coalfield Region of Kentucky (Pond and McMurray 2000 draft). This study adopted Kentucky's genus level Tentative Scoring Criteria for MBI Metrics (Table 3) and the Tentative MBI and Habitat Narrative Scoring Criteria (Table 4).

Table 3. Genus level tentative scoring criteria for MBI metrics from Pond and McMurray (2000, unpublished).

METRIC	SCORE		
	6	3	0
Taxa Richness	>40	20 - 39	<20
EPT Index	>22	11 - 22	<11
Biotic Index	<2.68	2.68 - 4.50	>4.51
% Clingers	>50	25 - 50	<25
% Ephemeroptera	>43	22 - 43	<22
% Chironomidae + Oligochaeta	<3.0	3.1 - 7.4	>7.4

Table 4. Genus level tentative MBI and habitat narrative scoring criteria (genus level) from Pond and McMurray (2000 unpublished).

Metric	Narrative Scoring Criteria			
	Excellent	Good	Fair	Poor
MBI	33 - 36	27 - 30	18 - 24	0 - 15
Habitat score	175 - 200	161 - 174	147 - 160	0 - 146

To provide a unitless and weighted scoring method, the actual result for each metric (Table 5) was given a score of 6, 3, or 0 (from Table 3). The metric scores were then summed to yield the MBI (Table 5). Habitat and MBI narrative rankings (excellent, good, fair, poor) were derived from Table 4.

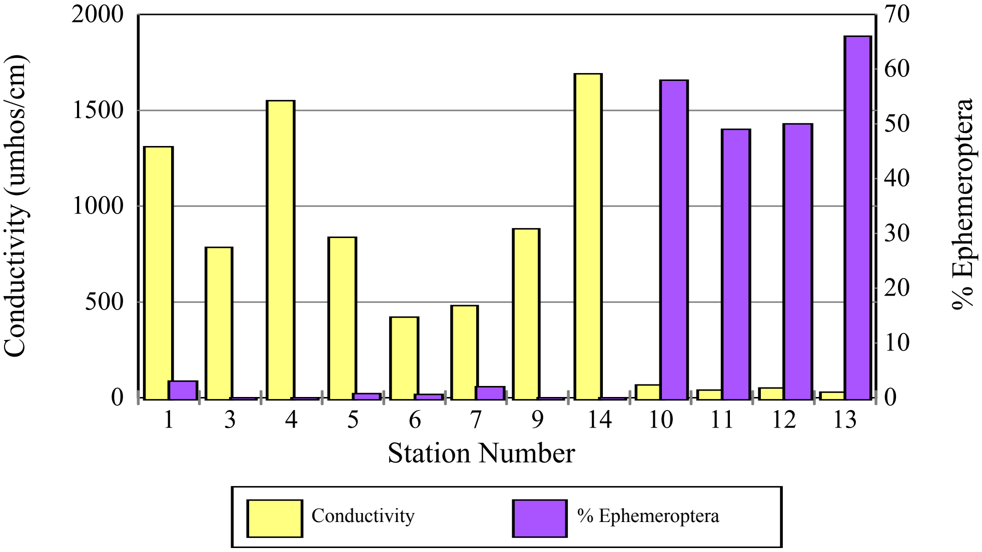
Table 5. Metric results, tentative scores, and final index (MBI) scores, Eastern Kentucky. May 1-4, 2000.

Station	METRIC RESULTS AND TENTATIVE SCORES						HABITAT		MBI	
	Taxa Richness	EPT Index	Biotic Index	% Clingers	% Ephem	% Chir + Olig	Score	Rank	Score	Rank
1	34	12	4.19	54	3	26	173	good	15	poor
3	24	4	5.56	3	0	92	166	good	3	poor
4	31	9	5.46	13	0	81	128	poor	3	poor
5	42	11	3.74	28	0.77	50	138	poor	15	poor
6	28	15	4.42	22	0.57	54	144	poor	9	poor
7	33	9	5.52	3	2	83	131	poor	3	poor
9	31	4	4.86	7	0	85	171	good	3	poor
14	25	7	4.92	21	0	38	149	fair	3	poor
10 (ref)	46	21	3.23	59	58	2	167	good	30	good
11 (ref)	38	16	3.23	46	49	3	174	good	24	fair
12 (ref)	41	24	2.97	29	50	3	181	excellent	30	good
13 (ref)	47	24	2.74	59	66	4	181	excellent	30	good

(ref) - reference watershed

Of the individual core metrics, % Ephemeroptera (Table 5) revealed the greatest sensitivity to environmental perturbation. A composition measure, % Ephemeroptera, represents the numerical abundance of mayflies as a percentage of the total individuals collected at a site. Past studies by EPA Region 4 in the Martha Oil Field region of Kentucky (U.S. EPA 1989), Hurricane Creek in Alabama (U.S. EPA 2000c) and recent studies in West Virginia by EPA Region 3 (Green et al. 2000) have identified a strong correlation between elevated conductivity and low numbers of mayflies in streams where mining operations exist. Figure 2 depicts the inverse relationship between elevated conductivity and absence or paucity of Ephemeroptera noted at test sites in the present study. Mayflies, along with the stoneflies (Plecoptera) and caddisflies (Trichoptera) are generally considered pollution-sensitive macroinvertebrates. Mayflies were absent in samples collected at half (4) of the test sites. The remaining four test sites had % Ephemeroptera results ranging from only 0.57% to 3.0% (Table 5). Conversely, reference sites had % Ephemeroptera ranging from 49% to 66%.

Figure 2. Conductivity and % Ephemeroptera at reference and test sites.



Although mayflies were drastically reduced in streams associated with mountaintop mining operations, pollution-sensitive stoneflies (Plecoptera) and caddisflies (Trichoptera) were collected at those locations. The core metric EPT Index, a summation of taxa in the pollution-sensitive Ephemeroptera, Plecoptera, and Trichoptera, is a richness measure specifically focusing on the presence/absence of pollution-sensitive fauna. Although effects were not as severe as those observed in the mayfly fauna, comparison of the range of the EPT Index at the test sites (4 to 15) with that of the reference sites (16 to 24) indicated the loss of some stoneflies and caddisflies at the test sites (Table 5). The core metric EPT Index has been identified in past studies and in the literature as one of the most discriminatory metrics (Barbour et al. 1996; Wallace et al. 1996).

A third metric, Taxa Richness, is the sum of benthic macroinvertebrate species collected from a given stream location and represents diversity. Taxa Richness values revealed a reduction in the number of benthic macroinvertebrate species in test watersheds when compared to reference watersheds. For example, Taxa Richness at test sites ranged from 24 to 42 while Taxa Richness for the reference watersheds ranged from 38 to 47 (Table 5). The previously identified reduction in pollution-sensitive EPT fauna contributed to the decrease in Taxa Richness at the test sites.

The Biotic Index, derived from Hilsenhoff (1987), is calculated by applying tolerance values to collected individuals to derive a community-based estimate of overall pollution at a given site. The tolerance values of various taxa range from 0-10, with 0 being the most pollution intolerant and 10 being the most pollution tolerant taxa. The presence of sensitive (intolerant) organisms would result in a low Biotic Index value, whereas, the presence of more tolerant organisms would result in a higher value. Biotic Indices at the test sites were higher (3.74 to 5.56) than those at the reference watersheds (2.74 to 3.23).

Whereas the core metrics % Ephemeroptera and EPT Index focus on fauna sensitive to pollution, the % Chironomidae + Oligochaeta metric focuses on pollution-tolerant organisms. A composition measure, % Chironomidae + Oligochaeta represents the numerical abundance of pollution-tolerant midges

(Chironomidae) and worms (Oligochaeta) as a percentage of the total individuals collected at a site. In a healthy, balanced benthic macroinvertebrate community, percentages of pollution-tolerant organisms are minimal. This was not the case in watersheds associated with mountaintop mining operations. Percent Chironomidae + Oligochaeta at the test sites ranged from 26 to 92 (Table 5) with a mean of over 63%. Conversely, % Chironomidae + Oligochaeta for reference watersheds range from 2% to 4%.

Percent Clingers, the final core metric utilized in the derivation of the MBI, represents the numerical abundance of organisms (percentage of the total individuals) that are morphologically adapted for attachment to stream substrates in generally faster currents such as riffles. Percent clingers for watersheds associated with mountaintop mining operations ranged from 3 to 54 (Table 5) with a mean of 19; reference watersheds ranged from 29 to 59 with a mean of 48.

The MBI score, derived from all of the core metrics, ranged from 3 to 30. MBI scores at the reference sites ranged from 24 to 30 with three of the sites ranking in the “good” category and one site at the upper limit of the “fair” category (Table 5). All test sites associated with mountaintop mining ranked in the “poor” category based on the MBI results .

The discriminatory ability of the six core metrics is apparent in the box and whisker plots in Appendix B. These metrics exhibited no overlap in distributions in test versus reference watersheds, thus supporting their choice as strong discriminators of impaired and reference conditions and illustrating the severity of impairment in the benthic macroinvertebrate communities of watersheds associated with mountain top mining operations.

6.3 Habitat Evaluation

Habitat evaluation scores for watersheds associated with mountaintop mining ranged from 131 to 173 with a mean of 150, while reference sites ranged from 167 to 181 with a mean of 175 (Table 5, Appendix C). Four of the eight test sites had habitat evaluation scores in the “poor” category based on the KDOW criteria (Table 4). Habitat degradation, evidenced by the “poor” habitat characterization at these four test sites, was related to a decrease in the velocity/depth regime (habitat), moderate to severe embeddedness, and moderate to heavy sediment deposition. These test sites with “poor” habitat evaluation scores (Stations 4,5,6, and 7) also had MBI rankings in the “poor” category (Table 5). However, test sites with “good” habitat evaluation scores (Stations 1, 3, and 9) also had MBI rankings in the “poor” category. This suggests that factors other than habitat degradation may be involved in impairment of the benthic community at some locations. Habitat evaluation scores at two of the reference watersheds were in the “excellent” category while two were in the “good” category. In contrast to the test sites, the reference watersheds had MBI rankings in the “good” category with the exception of Station 11 which had an MBI ranking at the upper limit (24) of the “fair” category.

6.4 Substrate Size Characterization

Substrate size and composition were measured at eight of the 12 sampling sites. Following the sample design and analysis employed by EPA Region 3 (Green et al. 2000, Kaufmann et al. 1999, Bain et al. 1985), numeric values (e.g. Class Score, Table 6) were assigned to the substrate size classes. These class scores are proportional to the logarithm of the midpoint diameter of each size class (Kaufman et al. 1999).

Table 6. Substrate size classes and class scores, Kentucky Mountaintop Mining, May 2000.

Substrate Size Class	Size (mm)	Class Score
Bedrock	>4000	6
Boulder	>250 - 4000	5
Cobble	>64 - 250	4
Coarse Gravel	>16 - 64	3.5
Fine Gravel	>2 - 16	2.5
Sand	>0.06 - 2	2
Fines	<0.06	1

A mean substrate size class score (of the numerically transformed size class) was calculated for the sampling reach (Table 7). The reach level mean substrate size in millimeters was then calculated using the substrate size class score (Kaufmann et al. 1999). The median substrate size class or D_{50} was taken from cumulative % distribution graphs presented in Appendix D. The reach level percentages of sands and fines ($\leq$2mm diameter) were derived from the frequency of particles in these two size classes divided by the 55 total particle measurements. For example, if five of the measurements in the reach were classified as sand or fines, then the % of the substrate less than or equal to 2 mm would be 5/55*100 or approximately 9%.

Table 7. Summary of substrate size and composition data, Kentucky Mountaintop Mining, May 2000.

Substrate Parameter:	Reference (n=3)	Mined/Filled (n=5)
Mean substrate size class score and standard deviation	3.91 (0.52)	2.91 (0.30)
Calculated mean substrate size (mm) and substrate classification	141 (Cobble)	13 (Fine Gravel)
Median substrate size class or D_{50} (mm) and substrate classification	153 (Cobble)	21 (Coarse Gravel)
% substrate size $\leq$2 mm (sand and fines) and standard deviation	22.4 (6.4)	30.6 (9.1)

The substrate size data indicate that the mean substrate size class scores and the mean calculated substrate particle sizes were smaller in the mined sites than in the unmined sites (Table 7). The median substrate size class or D_{50} and the calculated mean substrate size yielded similar results (Tables 7 & 8).

The calculated mean substrate size and D_{50} of the reference sites included bed surface material that was generally characterized as cobble. The average percent substrate size ≤2mm (sands and fines) was 22.4 at reference sites and 30.6 at test sites. Substrate characterization metrics for individual stations are summarized in Table 8.

Table 8. Summary of substrate characterization metrics at sampling sites. May 2000.

Stream	Station	Median substrate size or D_{50} (mm)	Mean substrate size class score	Calculated mean substrate size (mm)	% ≤ 2mm (sands and fines)
Long Fork	1	11	2.57	5	36.4
Laurel Fork	4	13	2.65	6	40.0
Fugate Branch	5	30	3.31	26	21.8
Spring Fork/ Quicksand Cr.	7	35	3.04	14	34.6
Clemons Fork (ref)	10	350	4.43	295	21.8
Coles Fork (ref)	11	60	3.89	96	29.1
Big Double Cr. (ref)	12	50	3.40	31	16.3
Lick Branch	14	18	2.96	12	20.0

The median particle size at reference sites was characterized as large cobble whereas the median particle size at mined sites was characterized as coarse gravel.

7.0 Associations Between Benthic Macroinvertebrate Metrics and Physical/Chemical Variables

The physical and chemical conditions of the streams were described using direct measurements of *in situ* water quality, physical habitat, and substrate size and composition. Associations between the benthic metrics and conductivity, total habitat scores, sediment deposition scores, and percent sand and fines were explored with correlation analyses (Table 9) similar to methods employed by Region 3 (Green et al. 2000).

Table 9. Correlations between benthic macroinvertebrate metrics and physical/chemical variables. Values in bold are statistically significant at the p≤0.05 level.

r - correlation coefficient (p value)	Conductivity (µS/cm)	Habitat Score	Sediment Deposition Score	% ≤2mm (% sand and fines)
MBI	-0.71 **(0.009)**	0.60 **(0.038)**	0.47 (0.121)	-0.46 (0.251)
Taxa Richness	-0.64 **(0.024)**	0.38 (0.226)	0.23 (0.480)	-0.39 (0.337)
EPT	-0.72 **(0.008)**	0.47 (0.121)	0.38 (0.217)	-0.52 (0.188)
BI	0.68 **(0.016)**	-0.63 **(0.027)**	-0.46 (0.137)	0.63 (0.091)
% Chironomidae & Oligocheate	0.52 (0.085)	-0.60 **(0.038)**	-0.41 (0.184)	0.60 (0.119)
% Ephemeroptera	-0.77 **(0.003)**	0.65 **(0.022)**	0.53 (0.075)	-0.47 (0.233)
% Clingers	-0.38 (0.228)	0.55 (0.063)	0.35 (0.258)	-0.17 (0.685)
Conductivity		-0.48 (0.115)	-0.590 **(0.044)**	0.38 (0.354)
n=8 for % ≤ 2mm pairs, n=12 for all other pairs				

Generally, the benthic metrics responded as expected to the potential stressors. The MBI, Taxa richness, EPT, % Ephemeroptera, and % Clingers all decreased with increasing conductivity and increasing % sands and fines. While the metrics BI and % Chironomidae and Oligochaeta, identifying a lack of sensitive species and the presence of more tolerant species, was positively correlated with conductivity and % sands and fines.

The strong negative correlation between conductivity and % Ephemeroptera reaffirms the inverse relationship shown in Figure 2 (i.e., where conductivity is elevated, there is an absence or paucity of Ephemeroptera).

8.0 CONCLUSIONS

Measureable differences in pH, temperature, conductivity, and dissolved oxygen were observed between reference and test sites. The most noticeable difference was elevated conductivity observed at the watersheds associated with mountaintop mining operations. Average conductivity at the test sites was 21 times higher than at reference sites, suggesting conductivity as either a route by which impairment occurred in mined areas, or a surrogate for other factors that were not measured. A more comprehensive evaluation of stream water chemistry may provide information the would better explain stream impacts.

Habitat scores were correlated to several measures of diversity and dominance of key groups of macroinvertebrates. Habitat scores were generally lower at sampling locations downstream of test areas than at reference sites. In particular, active mining sites and recently mined sites received very poor sediment deposition and embeddedness scores (individual parameters within the RBP habitat evaluation), indicating increased sedimentation in streams associated with mining activity. Substrate characterization data also indicated that substrate particle sizes were smaller in the mined sites than in the unmined sites.

The core metrics used in this study proved to be strong discriminators of impaired and reference conditions. These metrics illustrated the severity of impairment in the benthic macroinvertebrate communities of watersheds associated with mountain top mining operations. Of the individual core metrics, % Ephemeroptera revealed the greatest sensitivity to environmental perturbation. A strong inverse relationship was apparent between elevated conductivity and absence or paucity of Ephemeroptera (mayflies) at the test sites. Mayflies were either absent or comprised < 3.0 % of the benthic community at the test sites. Conversely, reference sites had % Ephemeroptera ranging from 49% to 66%. Other metrics sensitive to perturbations, including EPT Index, Taxa Richness, and % Clingers, were generally lower at test sites than at reference sites. The biotic index and % Chironomidae + Oligochaete were higher at test sites, indicating the absence of sensitive species and the presence of more tolerant benthic organisms. These study results confirm that benthic macroinvertebrate communities at all the test sites were severely impaired. Specific responses of the benthic macroinvertebrate communities to mountaintop mining operations are expressed through a decrease in diversity, a reduction or absence of pollution-sensitive species (especially mayflies), and an increase in pollution-tolerant species.

Macroinvertebrate, habitat, and *in situ* water quality data collected during this study document significant differences between streams located in reference watersheds and streams located in watersheds with mountaintop mining/valley fill operations (test sites). Mining related sites generally had higher conductivity, greater sediment deposition, smaller substrate particle sizes, and a decrease in pollution sensitive macroinvertebrates with an associated decrease in taxa diversity compared to reference sites.

Recognizing that aquatic resources of a stream ecosystem are a reflection of its surrounding landscape and land uses (Minshall et al 1985), concerns arise when rugged, steep terrains covered by deciduous forest typical of the Central Appalachians are replaced by gently rolling hills and pastures. Non-woody organic matter, originating from densely-forested streams has been identified as the major energy base of aquatic ecosystems (Vannote et al. 1980, Cummins 1980, Merritt et al. 1984). Deforestation, an environmental liability associated with mountaintop mining operations, would naturally affect the organic inputs to the energy budgets of aquatic ecosystems. Disruptions in the biological processes of first- and second-order streams impact not only aquatic life within the stream, but also the functions that aquatic life contribute to downstream aquatic systems in the form of nutrient cycling, food web dynamics, and species diversity (Cummins 1980, Merritt et al. 1984).

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APPENDIX A:

Box and Whisker Plots

APPENDIX B:

Benthic Macroinvertebrate Identifications

APPENDIX C:

Habitat Evaluation Forms

APPENDIX D:

Substrate Characterization Data and Cumulative Distribution Graphs