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Downstream effects of mountaintop coal mining: comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools

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Abstract. Surface coal mining with valley fills has impaired the aquatic life in numerous streams in the Central Appalachian Mountains. We characterized macroinvertebrate communities from riffles in 37 small West Virginia streams (10 unmined and 27 mined sites with valley fills) sampled in the spring index period (March–May) and compared the assessment results using family- and genus-level taxonomic data. Specific conductance was used to categorize levels of mining disturbance in mined watersheds as low (<500 μS/cm), medium (500–1000 μS/cm), or high (>1000 μS/cm). Four lines of evidence indicate that mining activities impair biological condition of streams: shift in species assemblages, loss of Ephemeroptera taxa, changes in individual metrics and indices, and differences in water chemistry. Results were consistent whether family- or genus-level data were used. In both family- and genus-level nonmetric multidimensional scaling (NMS) ordinations, mined sites were significantly separated from unmined sites, indicating that shifts in community structure were caused by mining. Several Ephemeroptera genera (e.g., *Ephemerella*, *Epeorus*, *Drunella*) and their families (Ephemerellidae, Heptageniidae) were correlated most strongly with the primary NMS axis ($r > 0.59$ for these genera; $r > 0.78$ for these families). These same Ephemeroptera were absent and, thus, eliminated from most of the mined sites. Total Ephemeroptera richness and relative abundance both declined with increasing mining disturbance. Several other metrics, such as richness, composition, tolerance, and diversity, clearly discriminated unmined vs mined sites. Most family-level metrics performed well and approximated the strength of genus-based metrics. A genus-based multimetric index (MMI) rated more mined sites as impaired than did the family-based MMI. Water-quality variables related to mining were more strongly correlated to NMS axis-1 scores, metrics, and MMIs than were sedimentation and riparian habitat scores. Generally, the correlations between the genus-level MMI and water-quality variables were stronger than the correlations between the family-level MMI and those variables. Our results show that mining activity has had subtle to severe impacts on benthic macroinvertebrate communities and that the biological condition most strongly correlates with a gradient of ionic strength.

Key words: bioassessment, coal mining, macroinvertebrates, specific conductance, Ephemeroptera, multimetric index, taxonomic resolution.

Many studies have shown that coal mining activities negatively affect stream biota in nearly all parts of the globe (e.g., Lewis 1973a, b, Scullion and Edwards 1980, Winterbourn and McDiffett 1996, Garcia-Criado et al. 1999, Kennedy et al. 2003). Acidic coal mine drainage (pH $<$ 6) and associated water-quality degradation have been studied the most extensively of all effects (e.g., Herlihy et al. 1990, Maltby and Booth 1991, Winterbourn and McDiffett 1996, Verb and Vis 2000, Cherry et al. 2001, DeNicola and Stapleton 2002, Freund and Petty 2007). In the northern Appalachians and Allegheny Plateau, certain coal strata have higher S content than other strata and tend to cause acidic mine drainage. Some coal mining activities routinely produce acidic mine drainage, but mountaintop mining (MTM) in the steep terrain of the Central Appalachian coalfields of Kentucky, Virginia, and West Virginia generally results in alkaline mine drainage

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(pH > 7). Calcareous strata and lower concentrations of S in the coal help to explain this alkaline mine drainage. Coal is made up primarily of organic elements (e.g., C, H) and inorganic elements (e.g., Al, Fe, Ca, Mg, Na, K, and S), and it contains trace elements (including As, Be, Cd, Co, Cr, Hg, Mn, Ni, Pb, Sb, and Se).

During MTM, several overburden layers of sedimentary rock are removed to access coal layers. Some of the mined rock is returned to the mountaintop and graded, but excess spoil typically is placed in valleys adjacent to the surface mine, resulting in valley fills (VFs) or hollow fills (detailed in Slonecker and Benger 2002). VFs permanently bury the ephemeral, intermittent, and perennial streams located adjacent to the mining operations. Land reclamation involves regrading and revegetation using grasses and other herbaceous plants that might be exotic (e.g., *Lespedeza cuneata*). Unlike clear-cut logging, colonization by native plants and trees is normally very slow because of heavy removal of topsoil and compaction of remaining soils on mine sites (Handel 2003). Biogeochemical properties of reclaimed mine soils can be radically different from forest soils, especially in terms of C and nutrient availability (Simmons et al. 2008). Across the MTM region as a whole, Wickham et al. (2007) found that interior forest loss from MTM was 1.75 to 5× greater than overall forest loss attributable to MTM and indicated that fragmentation of forests and introduction of edge forest can change the condition and ecological function of the remaining forest.

The direct impacts of MTM and associated fills on buried streams are undisputed (USEPA 2005). The streams buried by the overburden are permanently eliminated, and MTM and associated VFs have several indirect effects on downstream waters. Precipitation and groundwater in the mined watersheds percolate through the unconsolidated overburden on the mined sites and in the VFs and dissolve minerals until they discharge from the toe of the fills as surface water. The water quality downstream of the VFs can have elevated levels of SO_4, Ca, Mg, hardness, Fe, Mn, Se, alkalinity, K, acidity, and NO_3/NO_2 (Bryant et al. 2002). Sediment runoff is controlled through a series of sediment-control structures and ponds, but excess fine sediment might be increased in streams downstream of VFs (Wiley and Brogan 2003). Moreover, decreased evapotranspiration on the mined site and storage in the VFs can increase instream baseflows 6 to 7× downstream of VFs compared to unmined streams (Wiley et al. 2001), and peak flows might be higher (Wiley and Brogan 2003). These water-quality, hydrological, and physical habitat changes have the potential to negatively affect the instream aquatic life downstream of alkaline MTM and the associated VFs.

Contemporary MTM effects on downstream benthic macroinvertebrates have been reported in West Virginia and Kentucky (Green et al. 2000, Chambers and Messinger 2001, Howard et al. 2001, Pond 2004, Hartman et al. 2005, Merricks et al. 2007). Green et al. (2000) used family-level data because the state monitoring and assessments were done at the family level, and data comparability with state regulatory decisions was an important consideration. Green et al. (2000) also recognized that the family-level assessments might be conservative in that they might underestimate impairment caused by mining. Howard et al. (2001) and Pond (2004) identified consistent impairment of VF streams using genus-level data in Kentucky.

The West Virginia Department of Environmental Protection (WVDEP), the state agency charged with protecting the state's waters under the Clean Water Act (CWA), currently uses the family-level Stream Condition Index (WVSCI; Gerritsen et al. 2000) to conduct bioassessments and interpret the effect as biological impairment of aquatic life use. The state has listed many of the streams located downstream of mined areas and associated VFs as impaired on their CWA section 303(d) list of waters needing Total Maximum Daily Loads (TMDLs) (WVDEP 2007b). In many instances, the mining activity and associated VFs are the only sources of pollutants in the watershed.

Despite these studies, there have been different interpretations by regulators, the regulated community, and researchers about the severity and potential cumulative effects of MTM on resident aquatic life (USEPA 2005). Disagreement between regulators and the regulated community concerning the severity of impairment from mining and VFs might stem from differences in level of taxonomic identification, the different analyses and metrics used by various entities (e.g., regulators, regulated community, and researchers), and the ways in which these metrics are used by state agencies to interpret compliance with water-quality standards. In the Central Appalachians, both West Virginia and Virginia state agencies use family-level assessments to assess stream conditions and all related stressors. However, US Environmental Protection Agency (EPA) Region 3 and WVDEP have recently developed a genus-level mulitmetric index (MMI) called the Genus-Level Index of Most Probable Stream Status (GLIMPSS; Appendix 1), and WVDEP is using this MMI to do assessments. Recent studies on the benefits of finer taxonomic resolution indicate more accurate assessments when genus- or species-level data are used rather than family-level data (Guerold 2000, Hawkins et al. 2000, Lenat and Resh 2001, Arscott et al. 2006), but family-level assessments are also useful (Bowman and Bailey 1998, Bailey et al. 2001, Pond and McMurray 2002, Chessman et al. 2007), and the choice of which to use depends on the objectives of the assessments. Here, we compare family- and genus-level data using regulatory tools, such as WVSCI and GLIMPSS, and selected metrics that are commonly used by states and the regulated community to determine attainment of aquatic life uses for CWA programs. We examine the severity of impairment in waters downstream of VFs using genus-level data and offer further analyses of correlated stressors.

Methods

Site selection and study area

We sampled a total of 27 mined sites with VFs (mined) and 10 unmined sites in the region of MTM in the Central Appalachians (ecoregion 69; Woods et al. 1996) of West Virginia (Appendix 2). We selected sites to provide a range of mining intensity and water quality typical of MTM in this ecoregion. Locations of sample reaches in mined sites ranged from 0.15 km to 2.2 km downstream of the nearest mainstem or tributary VF (mean = 0.8 km). These data spanned collections taken in 1999/2000 ($n = 19$ sites) and 2006/2007 ($n = 18$ additional sites). We evaluated 6 sites (3 reclaimed mined and 3 unmined) for temporal changes over a 6- to 7-y recovery period (1999/2000–2006/2007). We did not combine data from the 2006/2007 revisit samples from these 6 sites with data from other sites in any statistical tests, but we did include the data in exploratory analyses.

The ecoregion is characterized by highly dissected terrain with similar forest types, geology, and climate. Bedrock geology is sedimentary and consists of interbedded sandstones, siltstones, shale, and coal. The dominant vegetation is mixed mesophytic forest (Braun 1950). Most unmined sites had minor anthropogenic influences (e.g., roads, gas wells, past channelization, timbering). Therefore, we considered them to be least disturbed (Stoddard et al. 2006) rather than pristine or minimally disturbed. Mined sites were located downstream of VFs in perennial reaches. Whereas some mined sites had limited mining disturbance prior to the MTM (e.g., contour mining with no VFs), many sites were relatively undisturbed prior to mining. Site watershed areas were relatively small and ranged from 0.5 to 15 km^2. Small streams in this ecoregion typically flow through constrained valleys with relatively high gradients and have boulder–cobble substrates (Woods et al. 1996). Reach slopes in this study ranged from 2 to 7% with an average of 3% (USEPA, unpublished data). Precipitation patterns are generally uniform throughout the study region; however, in summer 1999, this coalfield region reached extreme drought status. Rainfall was considered to be normal in our study area during 2006/2007 sampling (US Drought Monitor Archives 2008; http://drought.unl.edu/dm/archive).

Macroinvertebrate data

We collected macroinvertebrates from riffles using a 0.5-m-wide kicknet (595-µm mesh) in the spring index period (March–May 1999/2000 and 2006/2007). Briefly, we composited 4 targeted 0.25-m^2 kick samples to obtain a 1-m^2 sample from a 100-m reach at each site. In the laboratory, we randomly subsampled organisms in gridded pans to obtain 200 ± 20% individuals. We identified individuals to the genus level for most groups, except Turbellaria, Nematoda, Hydracarina, and Oligochaeta. In cases where the number of sorted organisms was far greater than the target, we subsampled all samples to 200 organisms using a Fortran® program (http://129.123.10.240/WMCPortal/modelSection.aspx?section=125&title=build&tabindex=-1; Western Center for Monitoring and Assessment of Freshwater Ecosystems, Utah State University, Logan, Utah). We sorted entire samples for some sites with low densities. For family-level analyses, we collapsed genera and summed them to family names in the database.

Environmental data

Bryant et al. (2002) reported monthly water samples at our mined and unmined sites collected in 1999/2000 ($n = 19$ sites), but we sampled only 1 of the 18 remaining sites for water chemistry in 2007. We used mean ($n = 13$ mo) chemical concentrations for the sites sampled in 1999/2000, whereas the sample collected in 2007 consisted of a representative grab sample taken at the time of macroinvertebrate sampling. Chemical variables included total metals, dissolved Fe and Mn, nutrients (NO_3, total P), total suspended solids, alkalinity, hardness, anions and cations, pH, and specific conductance. We recorded in situ physicochemical variables (pH, specific conductance, and temperature) at the time of benthic sampling at all 37 sites with a portable multiparameter sonde (Hydrolab Quanta; Hydrolab Corp., Austin, Texas). Sample collection, analytical methods, and results for water chemistry (1999/2000 data set) were reported in Bryant et al. (2002).

Percent mining in the catchment might serve as an appropriate indicator of mining disturbance, but we thought that our mining land-cover estimates were not sufficiently accurate for quantification (e.g., outdated imagery and inaccurate satellite interpretation). We offer these estimates in Appendix 2 for information purposes only. SO_4 concentration has been recommended as a way to estimate mining disturbance in some studies (Herlihy et al. 1990, Rikard and Kunkle 1990), but we lacked SO_4 data for nearly ½ of the sites. Therefore, we assigned sites to 4 categories of mining disturbance (unmined, low, medium, high) using specific conductance as the indicator based on the strong relationship between monthly SO_4 and specific conductance in the Bryant et al. (2002) data set ($R^2 = 0.94$, $p < 0.001$, $n = 511$). Many studies have shown that specific conductance is also a strong indicator of land disturbance, such as urbanization or agriculture (Herlihy et al. 1998, Dow and Zampella 2000, Paul and Meyer 2001, Black et al. 2004), but our sites included only upstream mining disturbances. We derived mining disturbance categories by splitting the range of mined-site conductivities into 3 categories (low: <500 μS/cm [$n = 7$], medium: 500-1000 μS/cm [$n = 8$], high: >1000 μS/cm [$n = 12$]). These categories were used primarily for graphical interpretations and to interpret taxonomic composition along a categorical gradient.

We scored physical habitat (0–20 points/metric; 0–200 points for total score) at all sites using the US EPA Rapid Bioassessment Protocol (RBP) (Barbour et al. 1999). We considered only the following RBP habitat metrics, embeddedness, sediment deposition, channel alteration, riparian zone width, and the total score, based on our knowledge of these metrics in relation to mined watersheds and their overall responsiveness in these small Central Appalachian streams.

Data analyses

We ordinated family- and genus-level community composition data across all sites with nonmetric multidimensional scaling (NMS; PC-ORD, version 4.25; MjM Software, Gleneden Beach, Oregon) using the Bray–Curtis similarity coefficient (Bray and Curtis 1957, McCune and Grace 2002) based on $\log_{10}(x + 1)$ abundances. We computed the data with 400 maximum iterations, 40 real runs, and 50 randomized runs. We grouped sites as unmined or mined with low, medium, or high disturbance. Taxa found at <5% (~2 sites) of all sites were removed prior to running NMS (as recommended by McCune and Grace 2002). The final matrices included 88 genera (of 162 total) and 44 families (of 48 total). We also tested for congruence in genus- and family-level community composition with Mantel's test using matrices calculated from Bray–Curtis similarity matrices (McCune and Grace 2002). The Mantel test compared the 37-site Bray–Curtis matrices between the family- and genus-level data sets by testing the significance of the correlation between matrices using 1000 Monte Carlo permutations (McCune and Grace 2002). We used the nonparametric multiresponse permutation procedure (MRPP) to determine if genus and family composition differed between disturbance categories (PC-ORD). Ranked Sorenson distances from the 37 sites were used to test the hypothesis of no difference between categories. MRPP produced an *A*-statistic, which compared observed vs expected within-site homogeneity based on the distance matrices (positive *A*-values indicate higher within-site homogeneity than expected by chance, i.e., differences in invertebrate composition between sites), and a *p*-value indicating statistical significance.

We compared several commonly used macroinvertebrate metric values between unmined and all mined sites and analyzed the influence of family- and genus-level determinations on these comparisons. Metrics included genus- and family-level total taxon richness, Ephemeroptera–Plecoptera–Trichoptera (EPT) richness, Ephemeroptera richness, Plecoptera richness, biotic index (BI), and Shannon diversity (H′). Some of these metrics are component metrics of the WVSCI and GLIMPSS and some of them are used commonly by other entities (e.g., researchers and the regulated community). The BI indicates the abundance-weighted tolerance value of the subsample and relies on tolerance values used by WVDEP that correspond to values reported in Hilsenhoff (1988), Lenat (1993), and Barbour et al. (1999). We used *t*-tests (after confirming that metric skew was $<\pm 1$) to detect differences between unmined and all mined sites with genus- and family-level metrics.

We calculated family-level (WVSCI; Gerritsen et al. 2000) and genus-level (GLIMPSS; Appendix 1) MMIs. These MMIs are used by WVDEP to assess condition and aquatic life-use attainment throughout the state. A comparison of the component metrics is shown in Appendix 1. Briefly, GLIMPSS is calibrated by region and season, whereas WVSCI is applied statewide within a broad single index period. Both MMIs were developed using similar methods, the same reference-site selection criteria, and 100-point best standard value (BSV) scoring procedures (Barbour et al. 1999). WVDEP has established an impairment threshold at the 5th percentile of WVDEP's reference distribution. Sites that score at or above this threshold are considered not impaired, whereas sites that score below the threshold are considered impaired. We used GLIMPSS scoring criteria for WVDEP's spring index period (March–May). For the GLIMPSS and WVSCI, scores <62 and <68, respectively, were rated impaired.

We also related ordination results (i.e., NMS axis-1 scores), biological metrics, and MMIs to chemical and habitat data using Spearman correlation coefficients. We correlated water-quality concentrations to individual metrics and MMIs in a separate analysis because the (nearly) full suite of chemical variables was available for only 20 of the 37 sites. Distance (km) downstream of VFs and the total count of fills upstream of the sampling reach were correlated to biological indicators, but only within the mined-site data set.

Results

Assemblage comparisons

NMS produced 2-dimensional ordinations with relatively high resemblance between family- and genus-level determinations (Fig. 1A, B). A 2-dimensional solution was found with satisfactory stress values of 15.7% for the genus ordination and 18.1% for the family ordination. NMS axis 1 represented the most variance in both taxonomic treatments (45% for genus, 67% for family). Both axes accounted for significantly more variance than would be expected by chance (Monte Carlo permutation test, $p = 0.02$, 50 permutations). In both genus- and family-level runs, mined sites were separated considerably from unmined sites in ordination space, which indicates that shifts in community structure were caused by mining intensity.

In general, low, medium, and high disturbance sites were similarly aligned along the primary axis in the 2 ordinations, but in a few instances, the mined-site cluster overlapped the unmined-site cluster (Fig. 1A, B). MRPP showed similar significant differences in genus- and family-level composition between all 4 disturbance categories (genus: $A = 0.38$, $p < 0.00001$; family: $A = 0.36$, $p < 0.00001$). McCune and Grace (2002) suggested that an A-value >0.3 indicates very high within-group homogeneity. For genus- and family-level taxonomy, within-group variability (distance) was lowest in unmined and low-disturbance sites and greatest in high-disturbance sites. Within the 3 mined categories, MRPP still showed significant differences in assemblage composition (genus: $A = 0.15$, $p = 0.0006$; family: $A = 0.21$, $p = 0.00003$) across disturbance categories. The Mantel test showed a strong positive correlation between family- and genus-level Bray–Curtis dissimilarity matrices and, thus, high overall similarity between family- and genus-level composition with respect to the sites (standardized Mantel statistic, $r = 0.82$, $p = 0.001$). Revisited sites

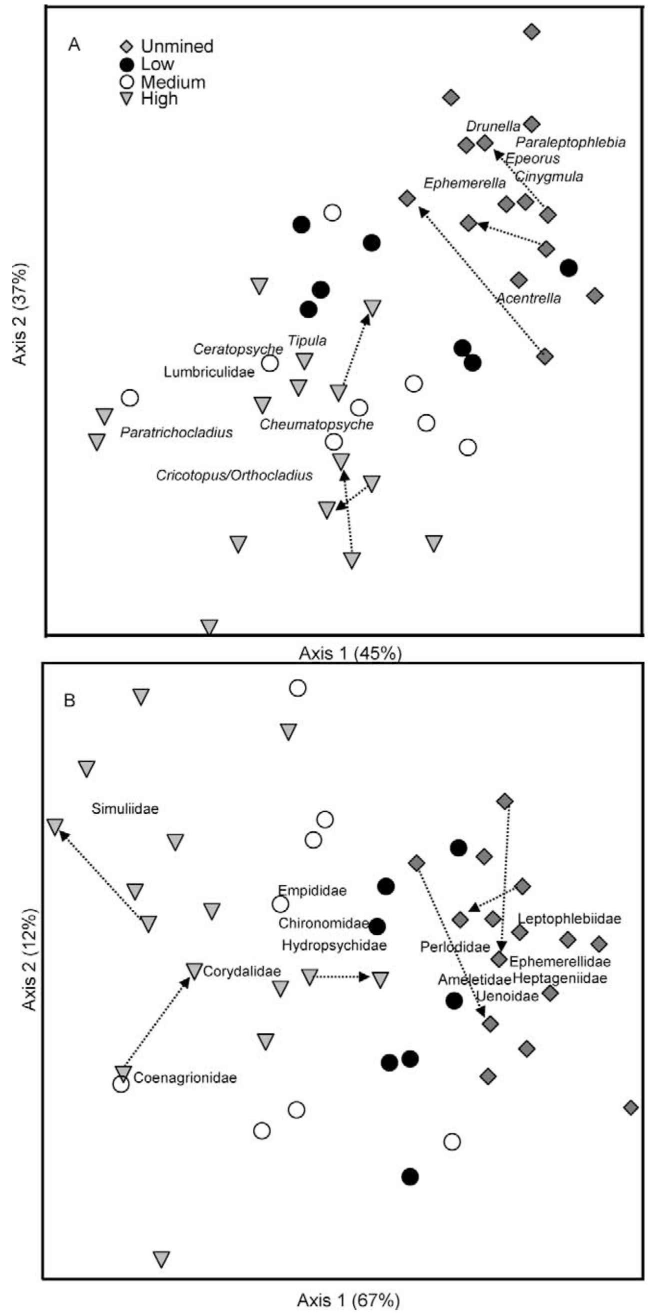

FIG. 1. Nonmetric multidimensional scaling ordination for genus (A) and family (B) determinations at sites categorized by mining disturbance (unmined, low, medium, high). Percent variance explained by each axis is in parentheses. Vectors represent temporal shift of community at 6 revisited sites from 1999/2000 samples to 2006/2007 samples. For clarity, only the 6 most strongly positively and negatively correlated taxa for axis 1 are shown.

(6–7-y period) shifted in ordination space (i.e., as indicated by vectors; Fig. 1A, B), but these pairs of sites generally plotted within their respective category domains.

TABLE 1. Mean metric values among unmined and mined sites. Statistical comparisons were based on Student's t-tests. EPT = Ephemeroptera, Plecoptera, Trichoptera.

Metric	Unmined	Mined	t	p
Total generic richness	31.9	21.7	–4.6	<0.001
Total family richness	19.9	11.7	–6.1	<0.001
EPT generic richness	17.9	8.9	–7.1	<0.001
EPT family richness	12.8	6.3	–5.9	<0.001
No. Ephemeroptera genera	8.2	2.1	–11.4	<0.001
No. Ephemeroptera families	4.7	1.6	–8.3	<0.001
No. Plecoptera genera	6.0	2.7	–5.3	<0.001
No. Plecoptera families	4.0	2.0	–4.2	<0.001
Genus Biotic Index	2.4	4.5	5.8	<0.001
Family Biotic Index	3.4	4.3	3.6	0.002
Genus Shannon H'	2.7	2.1	–3.7	0.002
Family Shannon H'	2.1	1.5	–3.9	0.001
% Orthocladiinae	5.1	22.1	4.8	<0.001
% Chironomidae	13.5	27.1	2.0	0.056
% Ephemeroptera	45.6	7.4	–6.4	<0.001
% Plecoptera	23.8	27.3	0.5	0.63
% EPT	77.9	51.1	–3.2	0.003

Simultaneous ordination of taxa and sites showed key genera and families typical of unmined and mined streams (Fig. 1A, B). In general, Ephemeroptera taxa were consistently weighted toward positive NMS axis-1 scores and unmined sites, whereas hydropsychid caddisflies, several Diptera, and oligochaetes were aligned with mined sites. Genera with the 5 highest correlations to NMS axis-1 scores included the caddisfly *Cheumatopsyche* ($r = -0.72$) and *Ceratopsyche* ($r = -0.62$), and the mayfly *Epeorus* ($r = 0.70$), *Ephemerella* ($r = 0.67$), and *Drunella* ($r = 0.59$). In the family ordination, families with the highest correlation to NMS axis-1 scores included the mayflies Ephemerellidae ($r = 0.89$), Heptageniidae ($r = 0.78$), Leptophlebiidae ($r = 0.67$), the caddisfly Uenoidae ($r = 0.68$), and the dipteran family Chironomidae ($r = -0.61$). The relative frequencies of EPT taxa among disturbance categories are reported in Appendix 3.

Metric comparisons

Nearly all metrics were able to detect mining influence, and t-statistics were generally stronger for genus-level metrics, but some family-level metrics performed as well as or better than genus-level metrics (e.g., total family richness, family Shannon H'; Table 1). Metric values for unmined sites were significantly different from metric values at mined sites ($p < 0.001$), except % Plecoptera ($p = 0.63$) and % Chironomidae ($p = 0.056$). Both genus and family Plecoptera richness metrics performed well (Table 1). Performance of the % Chironomidae metric ($t = 2.0$) was improved by identifying midges to the subfamily level (% Orthocladiinae, $t = 4.8$). Total and EPT richness declined similarly and consistently as disturbance category increased (Fig. 2A, B).

The greatest difference between family- and genus-level metrics occurred with the BI, an abundance-weighted pollution-tolerance metric. Low family BI values (i.e., representing the abundance of more sensitive taxa at unmined sites) were compressed within a narrow range and changed little between low-disturbance and unmined sites, whereas the more responsive genus BI decreased from >3 at low-disturbance sites to 0 at unmined sites to reflect the greater abundance of more sensitive genera present in the unmined sites (Fig. 3). A more consistent relationship between the genus- and family-level values of metrics was apparent at higher values of BI (>~3.5).

Family- and genus-level MMI comparisons

The GLIMPSS and WVSCI were strongly correlated ($r = 0.90$, $p < 0.0001$), and both MMIs generally agreed by assessing unmined sites as unimpaired and highly disturbed sites as impaired (Fig. 4). However, WVSCI appeared to underestimate impairment for some low- and medium-disturbance sites (Table 2).

Water chemistry, physical habitat, and biological relationships

Most of the chemical and physical variables differed significantly between unmined and mined sites (Mann–Whitney test, $p < 0.05$; Table 3). Mean elevation and watershed area did not differ significantly between mined and unmined sites. Mean water temperature did not differ significantly between mined and unmined sites ($p = 0.97$), even though many of the

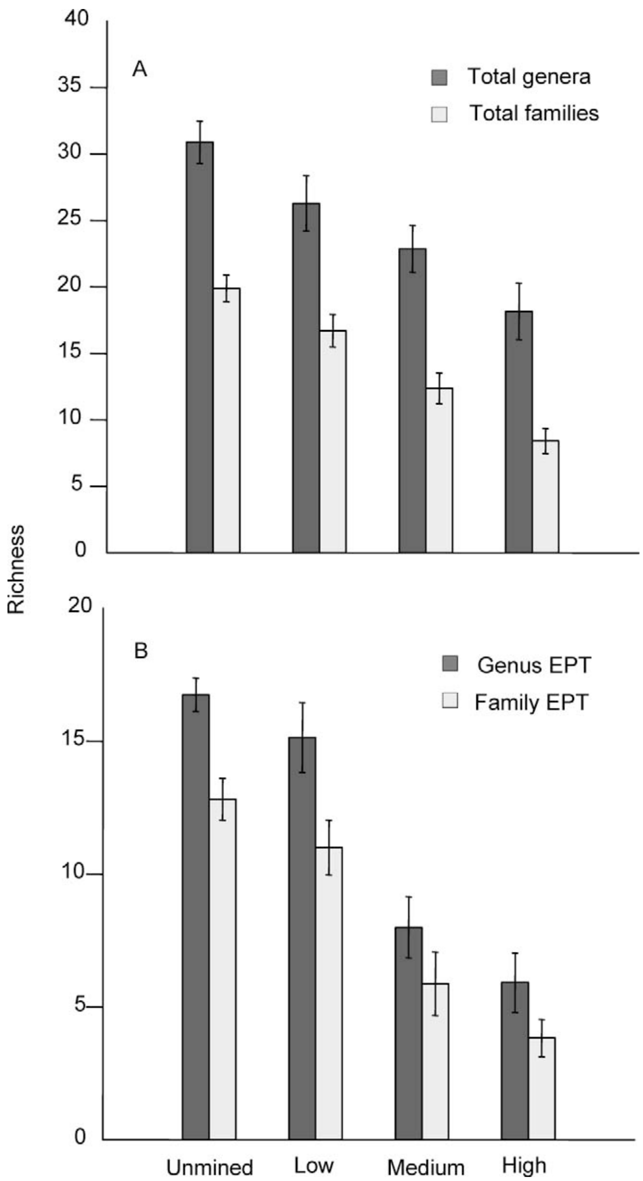

FIG. 2. Mean (±1 SE) total (A) and Ephemeroptera, Plecoptera, Trichoptera (EPT) (B) richness of genus- and family-level determinations across sites grouped by mining disturbance categories.

mined sites were downstream of sediment-control ponds, which can become warm from insolation. Measures of ionic strength, including individual ions, were more affected by mining than were individual metals or habitat metrics. We did not encounter classic acidic mine drainage because all of our mined sites had relatively high HCO_3 alkalinity and circumneutral pH.

For the 20-site subset, water-quality variables and the total RBP habitat scores were relatively strongly correlated with many biological metrics and the MMIs

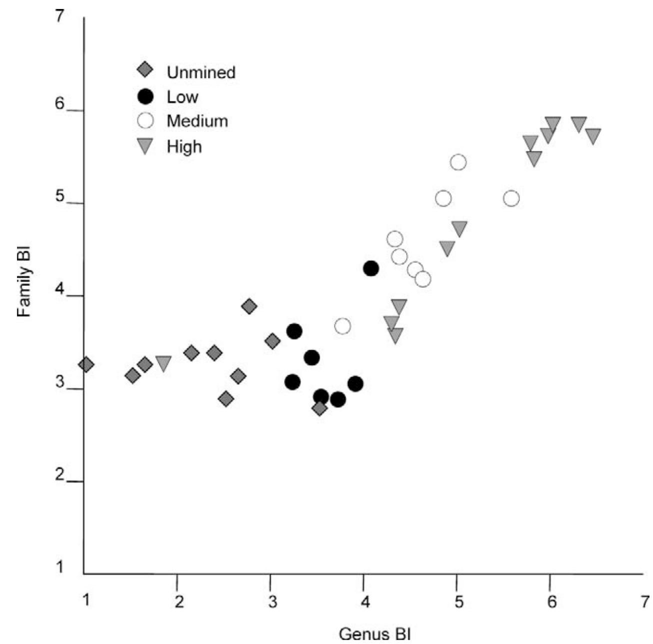

FIG. 3. Scatterplot for the relationship between genus and family biotic index (BI) values at sites categorized by mining disturbance.

(Table 4). Most biological metrics and the MMIs had substantially stronger correlations with specific conductance and individual ions than with the mining-related metals or individual habitat variables. NO_3-N was strongly correlated with many biological metrics, but total P was not detected at any site. The strongest relationships between biological variables and any metals were those between EPT and Ephemeroptera generic richness and Se ($r = -0.88$), and between % Chironomidae and dissolved Fe ($r = 0.61$).

For the complete 37-site data set and a smaller subset of environmental variables, the relationships between specific conductance and MMIs and NMS axis 1 were stronger than the relationships between pH, temperature, any of the individual habitat metrics, or the total RBP habitat score and MMIs or NMS axis-1 scores (Table 5). Percent Ephemeroptera showed a sharp nonlinear threshold response to specific conductance, whereby nearly all Ephemeroptera were eliminated from most medium- and high-disturbance sites (Fig. 5A, B). Percent Ephemeroptera was less strongly correlated with habitat-quality metrics than with specific conductance (see Table 4).

Temporal trends in condition

Minor shifts (i.e., vectors) in NMS ordination space (Fig. 1A, B) were observed for the 6 sites that were sampled in 1999/2000 and revisited in 2006/2007. After this 6- to 7-y period, both MMIs indicated that

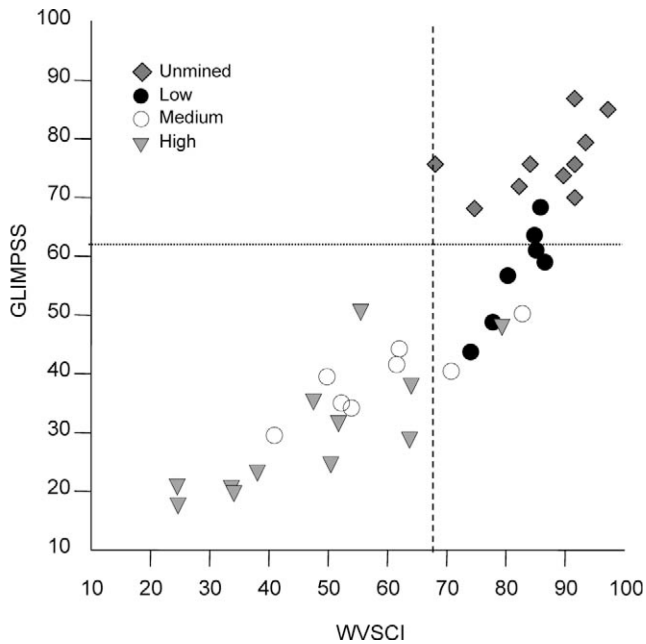

Fig. 4. Scatterplot for the relationship between Genus-Level Index of Most Probable Stream Status (GLIMPSS) and West Virginia Stream Condition Index (WVSCI) categorized by mining disturbance. Vertical and horizontal dashed lines represent impairment thresholds (68 for WVSCI; 62 for GLIMPSS) based on the 5th percentiles of West Virginia Department of Environmental Protection reference distributions.

the 3 mined sites remained impaired, and the sites showed variable signs of further degradation or slight improvement (Table 6). At Stanley Fork, MMIs, total taxon richness, EPT richness, and total RBP habitat score improved over time, but specific conductance increased substantially. MMIs at unmined sites were relatively stable or increased over time and indicated that unmined sites remained unimpaired. These sites were less variable than mined sites and had more consistent total taxon richness, EPT richness, and Ephemeroptera richness over time.

Discussion

The CWA directs states and tribes to designate beneficial uses for streams. Most waters in the US are designated for "aquatic life uses," which means the water must support fish, shellfish, insects, and other wildlife that inhabit the water. Water-quality standards, including numeric parameter-specific criteria and narrative criteria, are meant to protect those designated uses and specific aquatic life. Numeric water-quality criteria (e.g., Se, Fe, Al, pH, total suspended solids [TSS]) are sometimes exceeded in mined streams, but biological assessments (including

Table 2. Frequencies of assessment ratings of impaired and unimpaired based on genus- and family-level metrics for sites in 4 mining disturbance categories. GLIMPSS = Genus-Level Index of Most Probable Stream Status, WVSCI = West Virginia Stream Condition Index.

	GLIMPSS (genus level)		WVSCI (family level)	
Category	Impaired	Unimpaired	Impaired	Unimpaired
High	12	0	11	1
Medium	8	0	6	2
Low	5	2	0	7
Unmined	0	10	0	10

MMIs) more commonly indicate impairment. For example, Ephemeroptera are a major component of the macroinvertebrate assemblage and often account for 25 to 50% of total macroinvertebrate abundance in least-disturbed Central Appalachian streams sampled in the spring. Therefore, Ephemeroptera richness and composition metrics are appropriate indicators for bioassessments in this region. Our finding that entire orders of benthic organisms (e.g., Ephemeroptera) were nearly eliminated in MTM streams is a cause for concern and is evidence that the aquatic life use is being impaired.

Our results indicate that MTM is strongly related to downstream biological impairment, whether raw taxonomic data, individual metrics that represent important components of the macroinvertebrate assemblage, or MMIs are considered. The severity of the impairment rises to the level of violation of water-quality standards (WQS) when states use biological data to interpret narrative standards. For example, in West Virginia, the narrative WQS reads, "… no significant adverse impact to the chemical, physical, hydrologic, or biological components of aquatic ecosystems shall be allowed" (WVDEP 2007a). Pertinent to ionic stress effects, Kentucky's narrative WQS states, "Total dissolved solids or specific conductance shall not be changed to the extent that the indigenous aquatic community is adversely affected" (KYDEP 2007). Both WVDEP and Kentucky Department of Environmental Protection (KYDEP) have used biological data to interpret its narrative WQS and then listed mining-impaired streams on their 303(d) lists. More research is necessary to determine whether MTM-impaired streams can be restored to full-attainment status through water-quality improvements (e.g., permits or TMDL implementation) and physical restoration. Family-level assessments might detect high and moderate mining impacts and potential recovery endpoints, but we think that genus-level assessments will be required for thorough stressor

TABLE 3. Chemical and habitat variables at mined and unmined sites. Chemical values are in mg/L unless otherwise specified; p values are associated with comparisons between mined and unmined sites done with Kruskal–Wallis 1-way analysis of variance using the Mann–Whitney U-statistic. Total P was not detected in any samples (0.05 mg/L detection limit). RBP = Rapid Bioassessment Protocol.

	Mined		Unmined		
Variable	n	Mean (range)	n	Mean (range)	p
Watershed area (km^2)	27	4.9 (0.5–15.9)	10	3.0 (0.8–7.0)	0.516
Elevation (m)	27	313.2 (230–500)	10	307 (259–421)	0.973
Temperature (°C)	27	11.7 (7.8–18.2)	10	12 (7.3–16.5)	0.682
pH (SU)	27	7.9 (6.3–8.9)	10	7.1 (6.1–8.3)	0.005
Specific conductance (μS/cm)	27	1023 (159–2540)	10	62 (34–133)	0.000
Embeddedness score	27	13.6 (3–18)	10	16.4 (12–19)	0.004
Sediment deposition score	27	13.4 (6–18)	10	14.8 (10–19)	0.229
Channel alteration score	27	14.7 (7–19)	10	16.8 (15–18)	0.011
Riparian zone width score	27	14.5 (7–20)	10	16.4 (9–20)	0.143
Total RBP habitat score	27	147.8 (126–171)	10	158.5 (141–168)	0.006
HCO_3	13	183 (10.7–501.8)	7	20.9 (6.1–35)	0.002
Al (μg/L)	13	96 (<50–272)	7	92.5 (<50–183)	0.380
Ba (μg/L)	13	41.1 (22–68)	7	39.6 (15–72)	0.692
Ca	13	137.5 (38–269)	7	7.5 (2.7–12)	0.000
Cl	13	4.6 (<2.5–11)	7	2.8 (<2.5–4)	0.022
Cu (μg/L)	13	2.6 (<2.5–3.4)	7	2.9 (<2.5–5)	0.496
Hardness	13	801.4 (225–1620)	7	42 (17–72)	0.000
Dissolved Fe (μg/L)	13	91.8 (<50–281)	7	74.3 (<50–185)	0.362
Total Fe (μg/L)	13	275.6 (66–650)	7	176 (65–471)	0.322
Pb (μg/L)	13	1.2 (<1–4)	7	1.2 (<1–2.1)	0.496
Mg	13	122.4 (28–248)	7	4.3 (2.3–7)	0.000
Dissolved Mn (μg/L)	13	113.4 (6.5–853)	7	20.9 (<5–55)	0.165
Total Mn (μg/L)	13	141.4 (9–904)	7	34.1 (<5–83)	0.143
Ni (μg/L)	13	14.2 (<10–59)	7	<10	0.287
NO_3-N	13	3.4 (0.8–16.5)	7	0.4 (0.1–0.9)	0.001
K	13	9.9 (3–19)	7	1.6 (1.3–2)	0.000
Se (μg/L)	13	10.6 (<1.5–36.8)	7	<1.5	0.001
Na	13	12.6 (2.6–39)	7	2.4 (0.7–5.5)	0.001
SO_4	13	695.5 (155–1520)	7	16 (11–21.6)	0.000
Zn (μg/L)	13	9.1 (<2.5–27)	7	10.2 (3.3–23.4)	0.322

identifications and to detect subtle improvements from stressor abatement activities.

Our results confirm that MTM impact to aquatic life is strongly correlated with ionic strength in the Central Appalachians, but habitat quality did explain some variance in MMIs and other metrics. All mined sites with specific conductance >500 μS/cm were rated as impaired with the genus MMI (GLIMPSS). Undisturbed streams in the Central Appalachians are naturally very dilute, with background conductivities generally <75 μS/cm. Downstream of MTM sites, specific conductance and component ions can be elevated 20 to 30× over the background levels observed at unmined sites (e.g., SO_4: 38×, Mg: 32×, HCO_3: 15×) (Bryant et al. 2002). Mount et al. (1997) recognized the toxicity of major ions and developed predictive models to assess the acute toxicity attributable to major ions using *Ceriodaphnia dubia*, *Daphnia magna*, and *Pimephales promelas*. They reported that the relative ion toxicity was $K > HCO_3 \approx Mg > Cl > SO_4$; this order was confirmed by Tietge et al. (1997), who used the models to quantify and predict the toxicity from major ions but also identified toxicity from other toxic compounds in some high-salinity waters. Our data showed that the toxic ions reported by Mount et al. (1997) had strong correlations with benthic macroinvertebrate metrics and MMIs (Table 4) but at concentration ranges much lower than those reported in Mount et al. (1997).

Merricks et al. (2007) reported sporadic acute toxicity to *C. dubia* and *D. magna* at sites draining VFs in West Virginia but did not conclude whether ions or metals were responsible. Soucek and Kennedy (2005) observed SO_4 toxicity to *Hyallela azteca* but at higher concentrations (>2000 mg/L in hard water) than were found in our study. Our mined sites averaged nearly 700 mg/L SO_4, whereas unmined sites averaged only 16 mg/L. A water-quality guideline of <100 mg/L SO_4 was recommended to protect freshwater organisms in British Columbia (Singleton

Table 4. Spearman correlation coefficients ($n = 20$) between genus and family metrics and multimetric indices (MMIs) in relation to environmental variables from the 20-site data subset. All chemical variables are total concentrations unless specified as dissolved. Units are as in Table 3. Biological metrics are abbreviated to order or family name. GLIMPSS = Genus-Level Index of Most Probable Stream Status, WVSCI = West Virginia Stream Condition Index, RBP = Rapid Bioassessment Protocol. Temperature, total P, Ba, Cu, total Fe, Ni, Pb, and Zn were not significantly correlated with metrics ($p > 0.05$). Coefficients in bold are statistically significant ($p < 0.05$).

Variable	GLIMPSS	WVSCI	Total generic richness	Total family richness	EPT generic richness	EPT family richness	No. Ephemeroptera genera	No. Ephemeroptera families	No. Plecoptera genera	No. Plecoptera families
pH	–0.30	–0.29	–0.12	–0.36	–0.23	–0.30	–0.35	–0.37	–0.01	0.02
Specific conductance	**–0.90**	**–0.80**	**–0.74**	**–0.89**	**–0.88**	**–0.88**	**–0.90**	**–0.90**	**–0.75**	**–0.73**
Embeddedness score	**0.61**	**0.57**	**0.67**	**0.64**	**0.69**	**0.72**	**0.52**	**0.50**	**0.61**	**0.60**
Sediment deposition score	**0.52**	**0.62**	0.40	**0.50**	**0.56**	**0.66**	0.44	**0.52**	**0.47**	**0.45**
Channel alteration score	**0.51**	**0.46**	**0.58**	**0.50**	**0.52**	**0.53**	0.34	0.34	**0.58**	**0.58**
Riparian width score	0.21	0.04	0.37	0.21	0.24	0.22	0.13	0.08	0.26	0.23
Total RBP habitat score	**0.76**	**0.74**	**0.76**	**0.75**	**0.78**	**0.83**	**0.64**	**0.66**	**0.76**	**0.72**
HCO_3	**–0.78**	**–0.72**	**–0.67**	**–0.77**	**–0.76**	**–0.75**	**–0.75**	**–0.77**	**–0.65**	**–0.62**
Al	0.28	0.38	0.24	0.43	0.35	**0.45**	0.29	0.24	0.11	0.12
Ca	**–0.89**	**–0.79**	**–0.75**	**–0.86**	**–0.88**	**–0.87**	**–0.88**	**–0.89**	**–0.81**	**–0.75**
Cl	**–0.54**	–0.40	**–0.52**	**–0.57**	**–0.53**	**–0.52**	**–0.53**	**–0.48**	**–0.52**	**–0.50**
Hardness	**–0.89**	**–0.79**	**–0.74**	**–0.85**	**–0.89**	**–0.87**	**–0.87**	**–0.88**	**–0.81**	**–0.78**
Dissolved Fe	–0.29	–0.41	–0.04	–0.28	–0.30	–0.39	–0.33	–0.42	–0.22	–0.23
Mg	**–0.88**	**–0.83**	**–0.71**	**–0.85**	**–0.89**	**–0.90**	**–0.87**	**–0.89**	**–0.81**	**–0.79**
Dissolved Mn	**–0.46**	**–0.45**	–0.17	–0.34	–0.39	–0.43	**–0.46**	**–0.53**	–0.32	–0.30
Total Mn	–0.36	–0.35	–0.04	–0.25	–0.28	–0.35	–0.37	**–0.47**	–0.21	–0.20
$NO_2 + NO_3$	**–0.86**	**–0.82**	**–0.68**	**–0.83**	**–0.83**	**–0.79**	**–0.89**	**–0.87**	**–0.79**	**–0.75**
K	**–0.92**	**–0.88**	**–0.75**	**–0.89**	**–0.88**	**–0.90**	**–0.88**	**–0.89**	**–0.77**	**–0.72**
Se	**–0.85**	**–0.78**	**–0.76**	**–0.82**	**–0.88**	**–0.84**	**–0.88**	**–0.87**	**–0.83**	**–0.80**
Na	**–0.67**	**–0.57**	**–0.57**	**–0.68**	**–0.57**	**–0.59**	**–0.60**	**–0.59**	**–0.48**	**–0.42**
SO_4	**–0.89**	**–0.79**	**–0.75**	**–0.88**	**–0.89**	**–0.88**	**–0.89**	**–0.87**	**–0.79**	**–0.69**

2000). We think that surrogate test organisms (e.g., daphnids, amphipods) are more tolerant of pollutants than are resident Appalachian biota and that toxicity results might not translate into protective criteria.

Elevated conductivity can be toxic through effects on osmoregulation (Wichard et al. 1973, McCulloch et al. 1993, Ziegler et al. 2007). Aquatic insects, such as Ephemeroptera, have relatively high cuticular permeability and regulate ion uptake and efflux using specialized external chloride cells on their gills and integument and internally via Malpighian tubules (Komnick 1977, Gaino and Rebora 2000). Large increases in certain ions can disrupt water balance and ion exchange processes and cause organism stress or death. Tests for conductivity toxicity for mayflies have produced varying results (Goetsch and Palmer 1997, Chadwick et al. 2002, Kennedy et al. 2003, Hassell et al. 2006), but we think that these studies used taxa that are more tolerant (i.e., *Hexagenia*, *Centroptilum*, *Cloeon*, *Isonychia*) than Central Appalachian mayflies (e.g., ephemerellids, heptageniids). Other unknown effects might include ionic stress on reproductive success.

Even at relatively low concentrations, increased conductivity can cause significantly higher drift rates in benthos (Wood and Dykes 2002), but some taxa are not affected (Blasius and Merritt 2002). It is plausible that sensitive taxa are absent from mined streams because of this drift, but increased drift does not explain how recolonization is hindered. Alternatively, elevated specific conductance might simply be an indicator of mining disturbance, and other mining-related variables (e.g., metal concentrations) might be causing or contributing to the impairment. Our bioassessment indicators were not strongly correlated with dissolved or total metals concentrations in the water column, but these results do not rule out possible exposure to metals via dietary uptake (Gerhardt 1992, Buchwalter and Luoma 2005, Cain et al. 2006, Buchwalter et al. 2007) or microhabitat smothering by metal hydroxide precipitate (Wellnitz et al. 1994, USEPA 2005).

TABLE 4. Extended.

Variable	Genus Biotic Index	Family Biotic Index	Shannon H' (genus)	Shannon H' (family)	% Ortho-cladiinae	% Chiro-nomidae	% Ephemer-optera	% Plecoptera	% EPT
pH	0.34	0.36	–0.12	–0.26	**0.53**	0.31	–0.35	–0.13	–0.32
Specific conductance	**0.83**	**0.68**	**–0.83**	**–0.83**	**0.48**	0.26	**–0.88**	–0.19	**–0.68**
Embeddedness score	**–0.53**	**–0.45**	**0.62**	**0.56**	–0.02	0.21	0.44	**0.45**	**0.47**
Sediment deposition score	**–0.60**	**–0.57**	**0.48**	**0.56**	–0.34	–0.23	**0.48**	**0.49**	**0.63**
Channel alteration score	**–0.46**	–0.32	**0.48**	0.36	–0.05	0.24	0.28	0.42	0.35
Riparian width score	–0.08	0.00	0.27	0.12	0.25	**0.55**	–0.05	0.10	–0.05
Total RBP habitat score	**–0.70**	**–0.57**	**0.72**	**0.71**	–0.21	–0.02	**0.58**	0.38	**0.60**
HCO_3	**0.81**	**0.70**	**–0.62**	**–0.73**	**0.54**	0.37	**–0.75**	–0.26	**–0.74**
Al	–0.24	–0.02	0.41	0.37	–0.24	–0.15	0.24	–0.08	0.17
Ca	**0.85**	**0.73**	**–0.78**	**–0.81**	**0.47**	0.26	**–0.88**	–0.28	**–0.74**
Cl	0.41	0.33	**–0.58**	–0.40	0.00	–0.16	**–0.46**	–0.10	–0.22
Hardness	**0.85**	**0.72**	**–0.77**	**–0.80**	**0.47**	0.26	**–0.90**	–0.26	**–0.73**
Dissolved Fe	0.41	0.34	–0.22	–0.43	**0.45**	**0.61**	**–0.51**	0.14	–0.40
Mg	**0.87**	**0.76**	**–0.74**	**–0.81**	**0.52**	0.32	**–0.92**	–0.32	**–0.77**
Dissolved Mn	**0.46**	0.44	–0.40	**–0.49**	0.42	0.35	**–0.52**	–0.07	–0.41
Total Mn	0.41	0.41	–0.30	–0.35	0.40	0.31	**–0.49**	–0.09	–0.36
$NO_2 + NO_3$	**0.85**	**0.82**	**–0.63**	**–0.73**	**0.60**	0.39	**–0.90**	–0.44	**–0.79**
K	**0.91**	**0.74**	**–0.78**	**–0.87**	**0.58**	0.40	**–0.90**	–0.27	**–0.80**
Se	**0.79**	**0.70**	**–0.68**	**–0.73**	0.44	0.32	**–0.86**	–0.28	**–0.72**
Na	**0.71**	**0.57**	–0.44	**–0.56**	**0.60**	0.32	**–0.58**	–0.20	**–0.60**
SO_4	**0.83**	**0.69**	**–0.79**	**–0.80**	**0.48**	0.26	**–0.88**	–0.25	**–0.71**

MMIs were correlated with Se, but Se is considered relatively nontoxic to invertebrates, and this element is a greater concern for bioaccumulation in vertebrates than it is for toxicity in invertebrates (Lemly 1999, Hamilton 2004). Ingersoll et al. (1990) reported chronic Se toxicity to *D. magna* at concentrations $>$10 to 100$\times$ higher than those found in our study, but Halter et al. (1980) reported chronic toxicity (14 d) in *H. azteca* at levels $\sim$2$\times$ as high as our maximum concentration. A review by deBruyn and Chapman (2007) suggested that Se could cause sublethal effects to invertebrates at concentrations considered safe for fish and birds.

In cases where MTM activities resulted in smaller increases in ionic strength, we observed less-severe biological impairment. Within the mined site data set, we found no evidence that MMIs were significantly correlated with the number of VFs upstream or distance from the fill ($p > 0.05$), but these indicators appeared to be related to our inexact estimates of the amount of mining in the watershed. Aerial photos of these particular operations revealed that VFs were relatively small in size and intervening unmined tributaries probably offered some degree of dilution to our downstream sampling sites. For example, Dingess Camp had 1 small VF in its headwaters and 2 intervening unmined tributaries upstream of our sample reach. This site was rated unimpaired and had corresponding specific conductance of 423 μS/cm and total RBP habitat score of 160. However, medium- and high-specific-conductance sites contained either 1 large VF or multiple small VFs with no intervening unmined tributaries to provide dilution. This observation suggests that maintaining some unmined watersheds to provide adequate dilution immediately downstream of future MTM projects might be an effective way to protect downstream resources. These unmined watersheds also could act as refugia for maintenance of regional diversity and sources of recolonization for some species for reclaimed or restored reaches below VFs (e.g., Lowe et al. 2006). Future research should focus on the impairment mechanism and should include investigations of chronic effects on osmoreg-

Table 5. Spearman correlation coefficients of Genus-Level Index of Most Probable Stream Status (GLIMPSS) and West Virginia Stream Condition Index (WVSCI) and genus- and family-level nonmetric multidimensional scaling (NMS) axis scores vs a truncated list of environmental variables available for the entire 37-site data set. Values in bold are statistically significant ($p < 0.05$). RBP = Rapid Bioassessment Protocol.

			Genus		Family	
	GLIMPSS	WVSCI	NMS 1	NMS 2	NMS 1	NMS 2
Temperature (°C)	0.09	0.02	–0.14	**0.33**	–0.02	**0.41**
pH (SU)	**–0.44**	**–0.47**	**–0.47**	**–0.39**	**–0.47**	0.01
Specific conductance (μS/cm)	**–0.91**	**–0.80**	**–0.84**	**–0.72**	**–0.90**	0.16
Embeddedness score	0.23	0.22	0.22	0.04	0.15	0.04
Sediment deposition score	0.20	0.28	0.30	–0.07	0.20	**–0.33**
Channel alteration score	0.29	0.20	0.28	0.15	**0.33**	0.00
Riparian score	0.11	0.02	0.15	0.04	0.14	0.19
Total RBP habitat score	**0.38**	**0.43**	**0.45**	0.12	**0.38**	–0.16
Watershed area (km^2)	–0.19	–0.19	–0.22	–0.26	–0.25	**–0.37**
Elevation (m)	0.16	0.24	0.07	0.30	0.15	0.29

ulation from elevated specific conductance, catastrophic drift with no recolonization, chronic metal exposure via dietary uptake, and further study of the most vulnerable life stages. It is necessary to identify the specific parameters causing impairment to develop appropriate water-quality standards and control solutions.

Influence of MTM and taxonomic resolution on community composition

Unmined streams had assemblages (genus and family level) that differed markedly from assemblages in mined streams; ordinations showed strong shifts in taxonomic composition as indicated by the spread of sites categorized by mining disturbance. In general, Ephemeroptera genera and families were most indicative of unmined streams and contributed the most to separation of sites in ordination space. Mined sites also revealed signature communities dominated by facultative and tolerant taxa such as orthoclads, hydropsychids, oligochaetes, and other Diptera. In both mined and unmined streams, Plecoptera abundance often was dominated by the nemourid *Amphinemura*, a moderately facultative genus that is ubiquitous in small streams throughout the ecoregion.

Use of genus or family taxonomic determinations did not affect our multivariate ordination interpretations. Lenat and Resh (2001) indicated that family-level data approximate finer taxonomic data with multivariate statistics (Furse et al. 1984, Bowman and Bailey 1998); however, Hawkins et al. (2000) found that genus-level multivariate predictive models performed better than family-level models in California streams. Arscott et al. (2006) also showed that genus- and species-level ordinations distinguished urban and agricultural impacts to streams better than did family-level ordinations in the Hudson River Valley. We reason that genus- and family-level ordinations were relatively similar in our data set because many families collected had few genera at each site and because of spatial proximity and physical similarity of small study streams within the ecoregion and strong chemical stressor effects on mined communities.

Metric comparisons

Condition assessment of aquatic resources should rely on proven indicator metrics that are responsive to increasing stress (Karr and Chu 1999). In our analyses, genus-level metrics most accurately detected mining impacts based on *t*-tests, but family- and order-level metrics also were highly successful. The fact that most family- and order-level metrics could easily discriminate mining influences confirms that VF sites were considerably impacted and would certainly represent nonattainment of CWA designated use for aquatic life. The commonly used % EPT metric was less sensitive than other metrics because this metric was driven primarily by the presence of tolerant hydropsychid caddisflies or *Amphinemura* at mined sites. Total and EPT richness was greatly reduced below VFs (by 30–50% of values at unmined sites). In contrast, Merricks et al. (2007) did not find a significant decline in taxon richness below VFs. However, the single reference site used by Merricks et al. (2007) had values of specific conductance that were 4× higher than the average value at our unmined sites, indicating some disturbance at their reference site. Furthermore, the taxon richness value at their reference site was only ½ of the taxon richness we commonly observed.

Genus-level data offer better responsiveness than family- or order-level data because of the larger number of taxa identified and the more accurate tolerance values assigned to genera (Lenat and Resh 2001, Chessman et al. 2007). Differences in total taxon richness might be minimized because generic richness within individual invertebrate families (i.e., low genus:family ratios) seems to be lower in small Central Appalachian streams than in larger warm-water systems. Therefore, family-level taxon richness offers a close approximation to genus-level taxon richness in these small Appalachian systems. Exceptions to this are, for example, the families Chironomidae, Baetidae, Ephemerellidae, Heptageniidae, Hydropsychidae, Elmidae, and Perlodidae. Some of these same genus-rich families contain genera with a wide range of pollution-tolerance values (Blocksom and Winters 2006). This fact was evident in the comparison of genus- and family-level BI values. The family-level BI will be less sensitive than the genus-level BI if genera within a family have a broad range of tolerance values. Genus and family BIs were better correlated in the mid- and upper range of the BI scale than in the low range, a result that might reflect the narrower range of tolerance values in the more pollution-tolerant families normally found in mined streams. Ephemeroptera metrics performed similarly across taxonomic levels (i.e., genus, family, and order levels); populations were nearly eliminated below VFs in our study and others (Howard et al. 2001, Pond 2004, Hartman et al. 2005, Merricks et al. 2007). The only mayflies observed frequently at our low- to medium-disturbance sites were *Baetis* and *Plauditus*, 2 relatively facultative genera (Appendix 3).

MMI comparisons and impairment ratings

Nearly all of the mined sites were assessed as impaired based on GLIMPSS, whereas none of the unmined sites was assessed as impaired. Assessment ratings based on genus- and family-level MMIs were in agreement 81% of the time for sites in our data set. Genus-level GLIMPSS assessment ratings also agreed with family-level WVSCI assessment ratings ~80% of the time during development of GLIMPSS, which included all forms of impacts (not just mining; WVDEP, unpublished data; $n = 421$ for spring index period, ecoregions 67–69; Woods et al. 1996). However, 18% of the time, the WVSCI missed moderate impairment as rated by the GLIMPSS (73 of 421 sites). We think that this discrepancy represents a significant loss in assessment accuracy and supports the use of genus-level assessments in all state regulatory assessments of stream condition and related stressors. Several authors have acknowledged that family-level assessments (MMIs, multivariate predictive models, pollution-tolerance indices, or ordinations) can detect obvious impairment in relation to reference conditions (Bailey et al. 2001, Lenat and Resh 2001, Arscott et al. 2006, Chessman et al. 2007). WVSCI detected severe impacts but missed some low- to moderate-disturbance impacts that were detected by the GLIMPSS. The limitations of the WVSCI are that it does not use Ephemeroptera metrics, which were part of the GLIMPSS, and it does not account for spatial and temporal differences in benthic communities, whereas GLIMPSS is specifically calibrated to reference sites that have been seasonally and regionally partitioned. We did not attempt to modify the WVSCI to account for these issues because seasonal or regional adjustments were considered unnecessary in the development of WVSCI (Gerritsen et al. 2000).

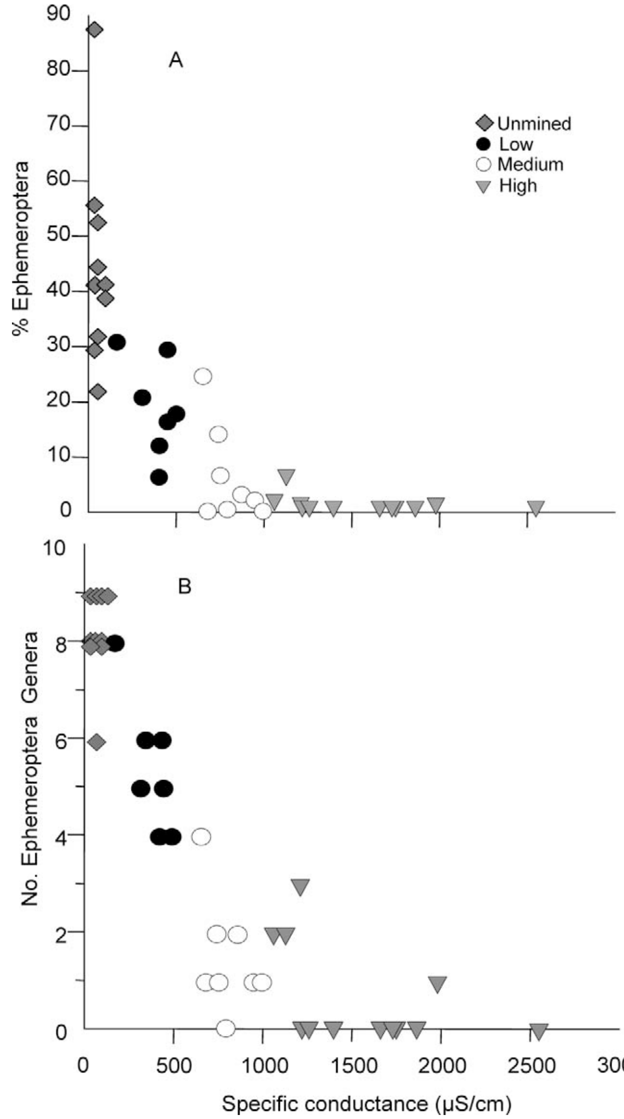

FIG. 5. Scatterplots for the relationship between % Ephemeroptera (A) and number of Ephemeroptera genera (B) and specific conductance at sites categorized by mining disturbance.

TABLE 6. Multimetric indices (MMIs), selected metric values, specific conductance, and total Rapid Bioassessment Protocol (RBP) habitat scores for 6 sites (3 reclaimed mined sites, 3 unmined sites) visited in 1999/2000 and revisited in 2006/2007. GLIMPSS = Genus-Level Index of Most Probable Stream Status, WVSCI = West Virginia Stream Condition Index, EPT = Ephemeroptera, Plecoptera, Trichoptera.

	Ballard (mined)		Stanley Fork (mined)		Sugartree (mined)		Rushpatch (unmined)		Spring (unmined)		White Oak (unmined)	
Stream	1999	2006	2000	2006	1999	2006	1999	2006	1999	2006	2000	2007
GLIMPSS	51	38	21	34	32	29	75	75	74	79	75	85
WVSCI	55	52	25	38	52	36	68	90	90	95	91	88
Total generic richness	33	20	14	28	22	20	42	40	33	37	32	30
EPT generic richness	12	9	2	6	4	4	17	19	17	21	17	20
Ephemeroptera generic richness	3	3	0	0	0	0	9	7	8	8	9	8
Specific conductance (μS/cm)	1201	1195	1387	2010	1854	1910	60	70	51	66	64	88
Total RBP habitat score	148	149	145	155	141	154	147	144	156	149	161	163

Water-chemistry, physical habitat, and biological relationships

Water quality structured benthic communities more than habitat quality. Our study and others (Chambers and Messinger 2001, Howard et al. 2001, Fulk et al. 2003, Pond 2004, Hartman et al. 2005, Merricks et al. 2007) suggest that specific conductance is the best predictor of the gradient of conditions found downstream of alkaline mine drainage and VF sites in the Central Appalachians. In previous studies, MMIs and Ephemeroptera metrics were strongly negatively correlated with instream specific conductance in West Virginia (Green et al. 2000, Chambers and Messinger 2001) and Kentucky (Howard et al. 2001, Pond 2004). Yuan and Norton (2003) found that Ephemeroptera richness was particularly sensitive to increasing specific conductance in the Mid-Atlantic Highlands. Black et al. (2004) reported that Ephemerellidae and Heptageniidae (the 2 primary mayfly families eradicated from our high-specific-conductance sites) had low specific-conductance optima in Pacific Northwest streams. In an analysis of West Virginia data, Fulk et al. (2003) confirmed that WVSCI scores were negatively correlated with individual and combined ion concentrations, but also with the concentrations of Be, Se, and Zn. Hartman et al. (2005) reported significantly lower densities of Ephemeroptera, Coleoptera, Odonata, noninsects, scrapers, and shredders ($p < 0.03$) in West Virginia VF streams compared to reference streams, but they also found that total abundance of all organisms was not substantially reduced in VF streams. Hartman et al. (2005) also reported that Ephemeroptera family richness was negatively related to specific conductance and that many of the richness metrics were negatively related to particular metals. Ephemeroptera are known to be sensitive to trace metals, especially in soft waters (Clements 2004), but we found that metal concentrations in the water column were not strongly correlated to Ephemeroptera (except Se) in our hard-water mined streams. Last, NO_3-N was significantly related to benthic metrics, but we did not visually observe excessive algal growth during the surveys. However, we cannot assume that diatom communities were not affected at these sites. Total P was probably limiting (it was below the 0.05 mg/L detection limit). Thus, most N probably was exported from the watershed and was autocorrelated with ionic strength.

Individual physical habitat variables and total RBP habitat score were more correlated with MMIs or individual metrics in the 20-site subset (Table 4) than in the full data set (Table 5). The discrepancy between habitat correlations in the 20-site subset vs the 37-site data sets probably arose because more mined sites had better habitat quality in the 37-site data set than in the 20-site subset. Howard et al. (2001) and Pond (2004) reported that habitat indicators (chiefly sedimentation and embeddedness) were strongly correlated with MMIs and particular metrics in Kentucky headwater streams. Surface mining can deliver excess sediment to watersheds (Starnes and Gasper 1995, Waters 1995, Chambers and Messinger 2001). We did not observe excessive sedimentation in our sampled reaches downstream of VF sediment-control ponds, but sediments might be transported and deposited farther downstream. Hartman et al. (2005) did not find significant differences in sedimentation below VFs after 5 to 20 y and speculated that after the initial pulse of sediments from mining operations, fine sediments might be sufficiently flushed from headwater reaches.

Observations on recovery

Our 3 revisits to sites downstream of reclaimed MTM and VFs revealed little sign of biological recovery (with MMIs or selected metrics) after 6 to 7 y, whereas communities within the 3 unmined catchments remained relatively stable. Habitat improvement was subtle at the downstream reaches of mined streams, but specific conductance remained very high, indicating that water chemistry is limiting recovery of these communities. Impacts to ecosystem structure and function (i.e., soil and water biogeochemistry, leaf decomposition, macroinvertebrates) remained after 15 y of recovery of a coal-mined watershed in Maryland (Simmons et al. 2008), and the oldest VF site in the data set given in Merricks et al. (2007) still had downstream specific conductance values >1200 μS/cm and no mayflies after 15 y. Further studies are needed to determine long-term recovery patterns of aquatic communities downstream of MTM and VFs.

Concluding Comments

We explored a causal link between MTM and biological degradation, and our data support the type of logical argument summarized by Beyers (1998) for establishing causal connections. Fore (2003) modified Beyers' 10 criteria and demonstrated causal links between human disturbance and biological condition in mid-Atlantic streams. The 10 criteria are: 1) strength, 2) consistency, 3) specificity, 4) temporality, 5) dose response, 6) plausibility, 7) experimental evidence, 8) analogy, 9) coherence, and 10) exposure. Eight of the 10 criteria were relevant for constructing a causal inference argument with our bioassessment data. We excluded specificity (because the bioassessment tools respond to many sources of degradation) and exposure (because we did not evaluate exposure indicators in affected organisms). Our data met 6 of the remaining 8 relevant criteria:

1. Ninety-three percent of the mined streams and none of the unmined streams were impaired using the preferred genus-level GLIMPSS, indicating the strength of the association.
2. The relationship between MTM and biological impairment has been confirmed by other investigators working in the Central Appalachians of West Virginia and Kentucky, indicating consistency.
3. Because our unmined sites were not impaired and were selected to be typical of least disturbed reference sites, these sites are representative of premining conditions in the watershed. We think it is reasonable to conclude that mining disturbance preceded the observed biological change (temporality).
4. Biological condition degraded in response to increasing mining disturbance, as measured by mining-related water-quality parameters, indicating dose response.
5. The premise that MTM causes downstream biological degradation is plausible given the wholesale landscape changes, hydrological alterations, and potential toxicants that are discharged. For example, elevated ionic strength can impair osmoregulation, which offers a plausible mechanism of impairment to macroinvertebrates.
6. Similar stressors cause similar effects to those found here (analogy). For example, diverse human activities (urbanization, oil- and gas-well drilling, road salting) that produce elevated ionic strength or landscape disturbance also are correlated with downstream impairment in empirical studies, and experimental toxicity testing has confirmed the toxicity of mining-related component ions.

We are currently conducting chronic toxicity testing experiments using surrogate organisms to provide experimental evidence that quantifies the toxicity of these mining effluents on downstream waters. These experiments will test the ambient downstream waters and synthesized waters that will mimic the ionic components of waters downstream of mines but will not contain any other potential toxicants (e.g., metals). The results of these experiments will help to provide more coherence between empirical and experimental evidence on the downstream chemical effects of MTM to aquatic life.

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APPENDIX 1. Additional information on development and application of the Genus-Level Index of Most Probable Stream Status (GLIMPSS).

The GLIMPSS was developed using best standard practices based on US Environmental Protection Agency (EPA) guidelines (Barbour et al. 1999) and Karr and Chu (1999) using >3000 statewide probabilistic and targeted sites (265 reference, 775 stressed) collected between 1998 and 2006. US EPA Region 3 and West Virginia Department of Environmental Protection (WVDEP) used widely recommended index calibration and validation techniques that included testing 36 different metrics for discrimination efficiency, redundancy, response to stressors, variability, and precision. Data were partitioned by bioregional (Allegheny Plateau, Central Appalachian–Ridge and Valley) and seasonal (winter, spring, summer) factors after examining nonmetric multidimensional scoring (NMS) ordinations and metric distributions. GLIMPSS metrics and scoring criteria were developed for 7 strata. For our study sites, we used metrics and final scoring criteria developed for the Mountain Spring stratum (i.e., spring index period/combined ecoregions 67 and 69). Table A1 compares the metrics used in the GLIMPSS to the family-level West Virginia Stream Condition Index (WVSCI).

TABLE A1. List of metrics used in the Genus-Level Index of Most Probable Stream Status (GLIMPSS) and the West Virginia Stream Condition Index (WVSCI). Metric scoring formulae indicate metric value (x) divided by the best standard value (BSV) that corresponds to the 5^{th} or 95^{th} percentile (depending on metric response direction) of West Virginia Department of Environmental Protection (WVDEP) data distribution for all sites in the spring index period, ecoregions 67/69 (for GLIMPSS), or within a broader March to October index period applied statewide (for WVSCI). The final GLIMPSS and WVSCI scores are calculated as the average metric score. Final GLIMPSS scores <62 were rated impaired; final WVSCI scores <68 were rated impaired. Intolerant richness is based on count of taxa with tolerance values <4. EPT = Ephemeroptera, Plecoptera, Trichoptera.

Metric	Scoring formula (value/BSV)100
GLIMPSS	
Total generic richness	$(x/41.5)100$
Intolerant richness	$(x/22.5)100$
Ephemeroptera generic richness	$(x/11)100$
Plecoptera generic richness	$(x/9)100$
Clinger generic richness	$(x/21.5)100$
Genus biotic index	$(10 - x)/(10 - 1.7)100$
% Ephemeroptera	$(x/53.5)100$
% Orthocladiinae	$(100 - x)/(100 - 0.7)100$
% 5 dominant genera	$(100 - x)/(100 - 47.2)100$
WVSCI	
Total family richness	$(x/22)100$
EPT family richness	$(x/13)100$
Family biotic index	$(10 - x)/(10 - 2.6)100$
% EPT	$(x/89.3)100$
% Chironomidae	$(100 - x)/(100 - 1.7)100$
% 2 dominant families	$(100 - x)/(100 - 37.3)100$

APPENDIX 2. General site information by mining disturbance category. Estimated percentage of the watershed mined, number of fills, and distance of site from nearest fill were determined using 1993 Landsat multiresolution land-cover data (for the 1999–2000 data set) and by digitized aerial photography with a 2006 mining permit boundary layer (2006–2007 data set). Distance to nearest fill (distance) is indicated as a mainstem (M) or tributary (T) fill. Mining activity was recorded as of the time of sampling. LF = left fork, UT = unnamed tributary, – = not applicable.

Site	Year	Watershed	Disturbance category	Watershed area (km^2)	Elevation (m)	% mining	No. of fills	Distance (km)	Mining activity
Rockhouse	1999	Coal	Medium	4.0	292.8	47	1	0.37 (M)	Inactive
Beech	1999	Coal	Medium	11.6	289.8	19	5	0.67 (T)	Inactive
LF Beech	2000	Coal	High	6.8	280.6	45	1	0.55 (T)	Active
Buffalo	1999	Coal	Medium	3.1	500.2	1	5	0.35 (T)	Inactive
Sandlick	2007	Coal	Low	11.4	245.4	9	2	0.96 (T)	Active
Laurel	2007	Coal	High	15.9	244.7	20	7	0.75 (T)	Active
Hughes	2000	Gauley	Medium	9.9	283.7	32	8	0.44 (T)	Inactive
Neff	1999	Gauley	Low	3.0	424.0	12	3	1.65 (T)	Inactive
Robinson	2007	Gauley	High	12.4	341.3	76	7	1.1 (T)	Active
Sugarcamp	2007	Gauley	Medium	5.2	328.2	31	2	2.2 (T)	Active
UT Twentymile1	2007	Gauley	High	0.8	331.1	85	1	0.51 (M)	Active
Boardtree	2007	Gauley	High	2.9	316.9	80	1	0.15 (M)	Active
Hardway	2007	Gauley	High	1.4	349.9	14	1	0.62 (M)	Active
Sugartree	1999	Guyandotte	High	1.9	256.2	50	2	0.08 (T)	Inactive
Stanley	2000	Guyandotte	High	4.5	250.1	65	6	0.32 (T)	Inactive
Ballard	1999	Guyandotte	High	6.2	260.8	17	8	0.93 (T)	Inactive
Cow	2000	Guyandotte	Low	1.3	439.2	1	1	1.02 (M)	Inactive
LF Cow	1999	Guyandotte	Low	3.2	353.8	13	2	0.61 (T)	Inactive
Hall	1999	Guyandotte	Medium	0.5	439.2	65	1	0.37 (M)	Inactive
Whitman	2007	Guyandotte	Low	2.8	324.9	41	1	1.2 (T)	Active
Ellis Camp	2007	Guyandotte	Low	1.0	274.3	49	1	0.99 (M)	Inactive
Winding Shoals	2007	Guyandotte	High	1.2	243.2	75	1	0.64 (M)	Inactive
Camp	2007	Guyandotte	Medium	1.5	335.8	35	2	0.85 (M)	Active
Righthand	2007	Guyandotte	Medium	12.4	230.1	17	6	0.89 (T)	Active
Slab	2007	Guyandotte	High	9.3	255.9	52	4	0.99 (T)	Active
Jims	2007	Tug Fork	High	1.3	250.2	57	1	0.60 (M)	Inactive
Dingess Camp	2007	Tug Fork	Low	2.1	280.4	16	1	1.8 (M)	Inactive
Oldhouse	1999	Coal	Unmined	1.8	317.2	–	–	–	Unmined
White Oak	2000	Coal	Unmined	2.7	350.8	–	–	–	Unmined
Trace	2007	Coal	Unmined	1.8	259.1	–	–	–	Unmined
Neil	1999	Gauley	Unmined	3.9	289.8	–	–	–	Unmined
Rader	2000	Gauley	Unmined	5.3	420.9	–	–	–	Unmined
Ash	2007	Gauley	Unmined	7.0	286.5	–	–	–	Unmined
UT Twentymile2	2007	Gauley	Unmined	0.8	279.8	–	–	–	Unmined
Spring	1999	Guyandotte	Unmined	1.4	274.5	–	–	–	Unmined
Rushpatch	1999	Guyandotte	Unmined	2.1	286.7	–	–	–	Unmined
Cabin	1999	Guyandotte	Unmined	4.7	265.4	–	–	–	Unmined

APPENDIX 3. Relative frequency (%) of Ephemeroptera, Plecoptera, and Trichoptera occurrences among mining disturbance categories. Note the strong dose response of many taxa along the mining disturbance gradient.

Order	Family	Genus	Unmined (n = 10)	Low (n = 7)	Medium (n = 8)	High (n = 12)
Ephemeroptera	Ameletidae	*Ameletus*	90	71	25	0
Ephemeroptera	Baetidae	*Acentrella*	60	43	25	8
Ephemeroptera	Baetidae	*Baetis*	70	71	63	17
Ephemeroptera	Baetidae	*Diphetor*	10	0	0	0
Ephemeroptera	Baetidae	*Plauditus*	10	43	13	25
Ephemeroptera	Ephemerellidae	*Drunella*	90	57	0	0
Ephemeroptera	Ephemerellidae	*Ephemerella*	100	86	25	8
Ephemeroptera	Ephemerellidae	*Eurylophella*	20	0	0	0
Ephemeroptera	Ephemeridae	*Ephemera*	20	0	0	0
Ephemeroptera	Heptageniidae	*Cinygmula*	80	43	0	0
Ephemeroptera	Heptageniidae	*Epeorus*	100	43	0	0
Ephemeroptera	Heptageniidae	*Stenacron*	30	0	0	0
Ephemeroptera	Heptageniidae	*Stenonema*	30	14	0	0
Ephemeroptera	Isonychiidae	*Isonychia*	0	14	0	0
Ephemeroptera	Leptophlebiidae	*Paraleptophlebia*	90	43	0	0
Plecoptera	Capniidae	Capniidae	10	0	0	0
Plecoptera	Chloroperlidae	*Alloperla*	0	14	0	0
Plecoptera	Chloroperlidae	*Haploperla*	30	14	25	0
Plecoptera	Chloroperlidae	*Sweltsa*	20	14	0	0
Plecoptera	Leuctridae	*Leuctra*	90	29	25	17
Plecoptera	Nemouridae	*Amphinemura*	100	100	88	75
Plecoptera	Nemouridae	*Ostrocerca*	10	0	0	0
Plecoptera	Nemouridae	*Prostoia*	10	43	13	0
Plecoptera	Peltoperlidae	*Peltoperla*	0	29	13	8
Plecoptera	Perlidae	*Acroneuria*	30	29	13	8
Plecoptera	Perlidae	*Eccoptura*	0	0	0	8
Plecoptera	Perlidae	*Hansonoperla*	10	0	0	0
Plecoptera	Perlodidae	*Clioperla*	0	0	0	8
Plecoptera	Perlodidae	*Diploperla*	0	14	13	8
Plecoptera	Perlodidae	*Isoperla*	20	29	25	25
Plecoptera	Perlodidae	*Malirekus*	10	0	0	0
Plecoptera	Perlodidae	*Remenus*	30	29	0	8
Plecoptera	Perlodidae	*Yugus*	60	14	0	0
Plecoptera	Pteronarcyidae	*Pteronarcys*	60	43	13	0
Plecoptera	Taeniopterygidae	*Taenionema*	0	14	0	0
Plecoptera	Taeniopterygidae	*Taeniopteryx*	10	0	13	0
Trichoptera	Glossosomatidae	*Agapetus*	10	0	0	0
Trichoptera	Glossosomatidae	*Glossosoma*	0	14	0	8
Trichoptera	Hydropsychidae	*Ceratopsyche*	10	43	38	50
Trichoptera	Hydropsychidae	*Cheumatopsyche*	0	86	88	92
Trichoptera	Hydropsychidae	*Diplectrona*	80	71	75	42
Trichoptera	Hydropsychidae	*Hydropsyche*	10	57	88	67
Trichoptera	Hydroptilidae	*Hydroptila*	10	0	0	8
Trichoptera	Hydroptilidae	*Ochrotrichia*	0	0	0	8
Trichoptera	Hydroptilidae	*Stactobiella*	0	0	13	0
Trichoptera	Lepidostomatidae	*Lepidostoma*	10	0	0	0
Trichoptera	Limnephilidae	*Pycnopsyche*	20	0	0	0
Trichoptera	Philopotamidae	*Chimarra*	0	29	13	33
Trichoptera	Philopotamidae	*Dolophilodes*	20	43	13	8
Trichoptera	Philopotamidae	*Wormaldia*	20	0	0	0
Trichoptera	Polycentropodidae	*Polycentropus*	30	29	13	8
Trichoptera	Psychomyiidae	*Lype*	10	0	0	0
Trichoptera	Rhyacophilidae	*Rhyacophila*	70	57	50	17
Trichoptera	Uenoidae	*Neophylax*	70	71	13	8