APPENDIX 1
Macroinvertebrates

SEP 2 4 2010

Abstract

Multiple analyses of macroinvertebrate data predict that the Spruce 1 project will have significant effects on stream biotic communities. This appendix reviews the available evidence concerning the expected effects of the proposed Spruce No. 1 mine project on the macroinvertebrate community of receiving streams. The biota of streams draining the proposed Spruce No. 1 site were compared with those draining the similar and nearby completed Dal-Tex operation. The results showed that some naturally-occurring taxa will be locally extirpated in the receiving streams and likely be replaced by pollution-tolerant taxa if mining and filling proceed. This conclusion is supported by direct comparison of mined and un-mined streams and using a regionally-derived observed/expected index. The appendix also includes a discussion of appropriate invertebrate metrics and data collection and analysis methods and explains why the Agency focuses on changes to sensitive taxa and community composition. These results are supported by the State of West Virginia's multimetric index (WVSCI), which also indicates that the magnitude and extent of degradation is likely to increase.

A1.1 Introduction

As previously described in the RD, macroinvertebrates are diverse in streams near the Spruce No. 1 project area. This appendix focuses upon survey results from streams directly affected by the project and includes Oldhouse Branch, Pigeonroost Branch, White Oak Branch, and Seng Camp Creek. Comparative data from adjacent mined streams including Beech Creek, Left Fork Beech Creek, Rockhouse Creek, and Spruce Fork are also discussed.

Because of their productivity and intermediate position in the aquatic food web, macroinvertebrates in small streams and headwaters play a critical role in the delivery of energy and nutrients downstream (Vannote et al. 1980). Most macroinvertebrate taxa complete 99% of their life cycle as immature larvae in streams. The Spruce No. 1 mine project will likely impact most of the sensitive species, as well as several common species, of mayflies (Ephemeroptera, or "E"), stoneflies (Plecoptera, or "P"), and caddisflies (Trichoptera, or "T") currently inhabiting the proposed impact streams through direct burial, as well as chemical loading of pollutants to receiving waters. These "EPT" taxa, as a group, are often considered sensitive to pollutants. Data from other mountaintop mining/vallyfills (MTM/VF) related studies (Green et al. 2000, Howard et al. 2001, Pond et al. 2008, Pond 2010) within this subecoregion show that many of these taxa will be extirpated, that is, become locally extinct. EPA (Pond et al. 2008, Appendix 3) reported that at least 10 different genera of mayflies were commonly extirpated from receiving streams of MTM/VF operations, as well as several common stonefly and caddisfly genera. Previous studies revealed strong negative relationships between macroinvertebrates, particularly mayflies, and surface coal mining in southern West Virginia and eastern Kentucky (Chambers & Messinger, 2000; Pond 2004; Hartman et al., 2005; Merricks et al., 2007; Pond et al., 2008, Pond et al., 2010).

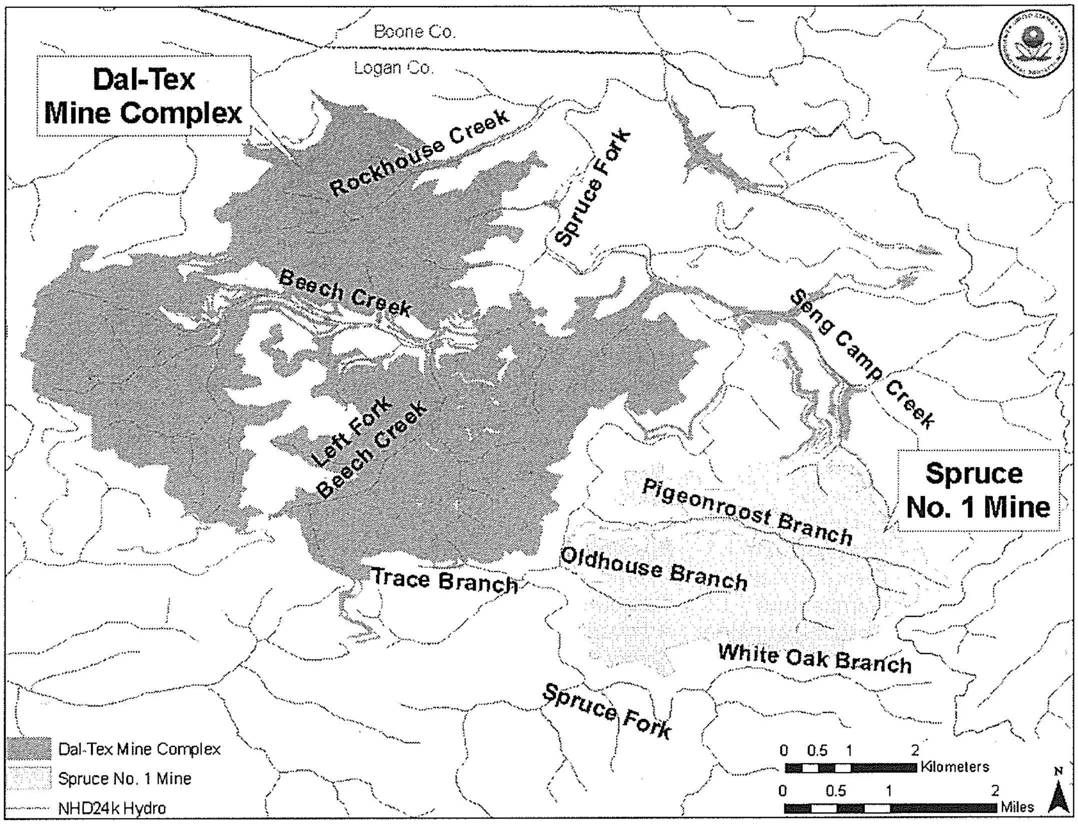


Figure 1. Map of Spruce No. 1 project area and adjacent Dal-Tex operation.

Table 1. List of all macroinvertebrate taxa identified from EPA sampling efforts at the Spruce project and Dal-Tex.

			Oldhouse +Pigeonroost	Beech+Left Fork Beech
Order	Family	Genus	Spruce No. 1	Dal-Tex Mine
Oligochaeta	Oligochaeta	Oligochaeta	X	X
Nematoda	Nematoda	Nematoda		X
Proseriataoela	Plagiostomidae	*Hydrolimax*	X	
Tricladida	Planariidae	Planariidae	X	
Basommatophora	Lymnaeidae	Lymnaeidae		X
Basommatophora	Physidae	Physella		X
Basommatophora	Planorbidae	*Helisoma*		X
Coleoptera	Dryopidae	*Helichus*	X	
Coleoptera	Elmidae	*Dubiraphia*		X
Coleoptera	Elmidae	*Macronychus*		X
Coleoptera	Elmidae	*Microcylloepus*		X
Coleoptera	Elmidae	*Optioservus*	X	X
Coleoptera	Elmidae	*Oulimnius*	X	X
Coleoptera	Psephenidae	*Ectopria*	X	
Coleoptera	Psephenidae	*Psephenus*	X	X
Decapoda	Cambaridae	*Cambarus*	X	
Diptera	Ceratopogonidae	*Atrichopogon*		X
Diptera	Ceratopogonidae	*Bezzia/Palpomyia*	X	X
Diptera	Ceratopogonidae	*Dasyhelea*	X	X
Diptera	Chironomidae	*Acricotopus*		X
Diptera	Chironomidae	*Chaetocladius*	X	X
Diptera	Chironomidae	*Corynoneura*	X	X
Diptera	Chironomidae	*Cricotopus*	X	X
Diptera	Chironomidae	*Diamesa*	X	X
Diptera	Chironomidae	*Eukiefferiella*	X	X
Diptera	Chironomidae	*Metriocnemus*		X
Diptera	Chironomidae	*Micropsectra*	X	X
Diptera	Chironomidae	*Microtendipes*	X	
Diptera	Chironomidae	*Orthocladius*	X	X
Diptera	Chironomidae	*Parachaetocladius*	X	
Diptera	Chironomidae	*Parametriocnemus*	X	X
Diptera	Chironomidae	*Paraphaenocladius*		X
Diptera	Chironomidae	*Paratanytarsus*		X
Diptera	Chironomidae	*Polypedilum*	X	X
Diptera	Chironomidae	*Rheotanytarsus*	X	X
Diptera	Chironomidae	*Smittia*		X
Diptera	Chironomidae	*Stempellinella*	X	
Diptera	Chironomidae	*Stenochironomus*		X
Diptera	Chironomidae	*Stilocladius*	X	
Diptera	Chironomidae	*Sympotthastia*	X	
Diptera	Chironomidae	*Tanytarsus*	X	
Diptera	Chironomidae	*Thienemanniella*		X
Diptera	Chironomidae	*Thienemannimyia*	X	X
Diptera	Chironomidae	*Tvetenia*	X	X
Diptera	Chironomidae	*Zavrelimyia*	X	
Diptera	Empididae	*Chelifera/Metachela*	X	X
Diptera	Empididae	*Clinocera*	X	
Diptera	Empididae	*Hemerodromia*		X
Diptera	Simuliidae	*Prosimulium*	X	
Diptera	Simuliidae	*Simulium*	X	X
Diptera	Tabanidae	Tabanidae		X
Diptera	Tipulidae	*Antocha*		X
Diptera	Tipulidae	*Cryptolabis*	X	
Diptera	Tipulidae	*Dicranota*	X	
Diptera	Tipulidae	*Hexatoma*	X	

Table 1. Continued.

Continued			Oldhouse +Pigeonroost	Beech+Left Fork Beech
Order	Family	Genus	Spruce No. 1	Dal-Tex Mine
Diptera	Tipulidae	*Limnophila*	X	
Diptera	Tipulidae	*Limonia*	X	X
Diptera	Tipulidae	*Pseudolimnophila*	X	
Diptera	Tipulidae	*Tipula*	X	X
Ephemeroptera	Ameletidae	*Ameletus*	X	
Ephemeroptera	Baetidae	*Acentrella*	X	
Ephemeroptera	Baetidae	*Baetis*	X	X
Ephemeroptera	Baetiscidae	*Baetisca*	X	
Ephemeroptera	Ephemerellidae	*Drunella*	X	
Ephemeroptera	Ephemerellidae	*Ephemerella*	X	
Ephemeroptera	Ephemerellidae	*Eurylophella*	X	
Ephemeroptera	Ephemeridae	*Ephemera*	X	
Ephemeroptera	Heptageniidae	*Cinygmula*	X	
Ephemeroptera	Heptageniidae	*Epeorus*	X	
Ephemeroptera	Heptageniidae	*Stenacron*	X	
Ephemeroptera	Heptageniidae	*Maccaffertium/Stenonema*	X	
Ephemeroptera	Isonychiidae	*Isonychia*	X	X
Ephemeroptera	Leptophlebiidae	*Paraleptophlebia*	X	
Megaloptera	Corydalidae	*Corydalus*		X
Megaloptera	Corydalidae	*Nigronia*	X	X
Odonata	Aeshnidae	*Boyeria*		X
Odonata	Gomphidae	*Lanthus*	X	X
Plecoptera	Capniidae	Capniidae	X	
Plecoptera	Chloroperlidae	*Haploperla*	X	
Plecoptera	Leuctridae	*Leuctra*	X	
Plecoptera	Nemouridae	*Amphinemura*	X	X
Plecoptera	Nemouridae	*Ostrocerca*	X	
Plecoptera	Nemouridae	*Prostoia*		X
Plecoptera	Peltoperlidae	*Peltoperla*	X	
Plecoptera	Perlidae	*Acroneuria*	X	
Plecoptera	Perlodidae	*Isoperla*	X	
Plecoptera	Perlodidae	*Remenus*	X	
Plecoptera	Perlodidae	*Yugus*	X	
Plecoptera	Pteronarcyidae	*Pteronarcys*	X	
Plecoptera	Taeniopterygidae	*Taenionema*	X	
Plecoptera	Taeniopterygidae	*Taeniopteryx*	X	X
Trichoptera	Glossosomatidae	*Agapetus*	X	
Trichoptera	Glossosomatidae	*Glossosoma*	X	
Trichoptera	Goeridae	*Goera*	X	
Trichoptera	Hydropsychidae	*Ceratopsyche*	X	
Trichoptera	Hydropsychidae	*Cheumatopsyche*	X	X
Trichoptera	Hydropsychidae	*Diplectrona*	X	X
Trichoptera	Hydropsychidae	*Hydropsyche*	X	X
Trichoptera	Hydroptilidae	*Hydroptila*		X
Trichoptera	Limnephilidae	*Pycnopsyche/Hydatophylax*	X	
Trichoptera	Philopotamidae	*Chimarra*	X	X
Trichoptera	Philopotamidae	*Dolophilodes*	X	
Trichoptera	Polycentropodidae	*Polycentropus*	X	
Trichoptera	Psychomyiidae	*Psychomyia*	X	X
Trichoptera	Rhyacophilidae	*Rhyacophila*	X	X
Trichoptera	Uenoidae	*Neophylax*	X	
Tricladida	Planariidae	Planariidae	X	
		Total Distinct Taxa	85	56
		Total EPT Taxa	42	12

Mayfly (Ephemeroptera) data from EPA, WVDEP, and the applicant's consultants (DEIS-Sturm Env. Services, BMI, Inc.) reveal that collectively, Pigeonroost Branch, Seng Camp Creek, and Oldhouse Branch contain a high number of sensitive mayfly taxa and individuals. A total of 20 taxa (Table 2) have been identified from these three headwater streams indicating these systems offer high water quality and optimal habitat. Many of these mayfly genera have not been collected in Spruce Fork, making these headwater streams distinctive in the permit area (those few taxa shared with Spruce Fork are generally facultative genera (not sensitive) such as *Baetisca, Baetis*, *Isonychia*). This list represents only an estimate of mayfly richness in these streams; several other genera have been found by WVDEP in other Spruce Fork tributaries and are potentially present in the project area.

Oldhouse Branch and Pigeonroost Branch represent an exceptionally high quality resource within the Spruce Fork watershed, providing refugia for aquatic life and potential sources for recolonizing nearby waters, which are experiencing significant stream degradation. As many as 9 mayfly taxa have been collected by EPA in Oldhouse Branch in any one season-specific sample (based on a random subsample of 200 organisms), with an average of 7 genera across multiple samples. This former observation ranks in the 95th percentile of 937 samples taken by WVDEP in the Central Appalachian ecoregion (also based on 200 organism subsamples). These data, given above, are significant and indicate that only 5% or less of the total number of streams in this ecoregion have more mayfly taxa than Oldhouse Branch. On a statewide scale (greater than 4000 samples), Oldhouse ranks in the 90th percentile. Pigeonroost Branch contained 8 genera in a season-specific sample, ranking it among the 90th percentile in the Central Appalachians and 83rd percentile statewide for single-sample observations indicating that these streams are refugia for aquatic life and potential sources for recolonizing nearby waters, and an exceptional, high quality resource within the Spruce Fork watershed which is experiencing significant stream impairment.

A recent study (Pond et al. 2008) found that mayfly richness is significantly reduced to a few or zero genera when conductivity exceeds 500 μS/cm below mining operations in West Virginia that are similar to the proposed action at Spruce No. 1. Table 2 shows which individual mayfly genera are found infrequently or are absent from MTM streams having greater than 500 μS/cm. The same mining-induced pattern was documented in the eastern Kentucky coalfields (Pond 2010).

Table 2. Presence/absence of mayfly genera in the permit area. Oldhouse, Pigeonroost and Seng Camp Creeks will be exposed to operations from the Spruce No. 1 Mine. Frequency of occurrence of particular mayfly taxa across 20 similar WV mine sites is presented for comparison. NA indicates that the taxon was not collected in Pond et al. (2008) study.

Order	Family	Genus	Oldhouse	Pigeonroost	Seng Camp	Frequency of Occurrence Below MTM/VF @ >500 µS/cm*
Ephemeroptera	Ameletidae	*Ameletus*	X	X		10%
Ephemeroptera	Baetidae	*Acentrella*	X	X		15%
Ephemeroptera	Baetidae	*Baetis*	X	X	X	35%
Ephemeroptera	Baetidae	*Diphetor*		X		0%
Ephemeroptera	Baetiscidae	*Baetisca*		X	X	NA
Ephemeroptera	Caenidae	*Caenis*			X	NA
Ephemeroptera	Ephemerellidae	*Attanella*		X	X	NA
Ephemeroptera	Ephemerellidae	*Dannella*		X	X	NA
Ephemeroptera	Ephemerellidae	*Drunella*	X	X		0%
Ephemeroptera	Ephemerellidae	*Ephemerella*	X	X		15%
Ephemeroptera	Ephemerellidae	*Eurylophella*	X	X	X	0%
Ephemeroptera	Ephemeridae	*Ephemera*	X	X	X	0%
Ephemeroptera	Heptageniidae	*Cinygmula*	X	X		0%
Ephemeroptera	Heptageniidae	*Epeorus*	X	X		0%
Ephemeroptera	Heptageniidae	*Heptagenia*		X		NA
Ephemeroptera	Heptageniidae	*Maccaffertium*	X	X	X	0%
Ephemeroptera	Heptageniidae	*Stenacron*	X		X	0%
Ephemeroptera	Isonychiidae	*Isonychia*		X	X	0%
Ephemeroptera	Leptophlebiidae	*Choroterpes*	X			NA
Ephemeroptera	Leptophlebiidae	*Paraleptophlebia*	X		X	0%

Note: *Siphlonurus* and *Pseudocloeon* reported by Sturm Env. are likely erroneous identifications[2]. These genera have been excluded from this list. * Based on samples from 20 MTM/VF sites Pond et al. (2008)

Stonefly data compiled from EPA, WVDEP, and the applicant's consulting firms show that Oldhouse, Pigeonroost, and Seng Camp collectively yielded 16 genera of stoneflies (Table 3). Oldhouse and Pigeonroost both had 11 total stonefly genera. A single collection in Oldhouse by EPA (Spring 2000) had 9 genera of stoneflies which ranks greater than the 98th percentile of all Central Appalachian streams sampled by WVDEP

---

[2] Region III conservatively excluded two mayfly genera from Table 2 which were identified by the permittee's consultant. *Siphlonurus* is exceedingly rare in this ecoregion and can be confused with *Ameletus*, a much more common inhabitant. The Region believes the identification may have been in error. Also, older taxonomic sources placed *Pseudocloeon* as *Acentrella* (formerly of the genus *Baetis*), and was eventually placed in the genus *Labiobaetis*. This genus does not frequent headwater streams like those in Oldhouse, Pigeonroost, and Seng Camp. The Region believes that it is most probable that previous records of *Pseudocloeon* are in fact, *Acentrella*.

(937 samples). This means that only 2% of stream samples in this ecoregion had more stonefly taxa than Oldhouse within a single sampling event. This makes Oldhouse remarkably diverse and distinctive in a watershed that has significant impairment. Pigeonroost Branch had 6 stonefly genera in any one season-specific sample (Spring 2000), ranking it at the 83rd percentile among 937 Central Appalachian streams. Similar to mayflies described above, stonefly survey data showed that stonefly richness is significantly reduced when conductivity exceeds 500 µS/cm in mining operations similar to Spruce No. 1. (Table 3).

Table 3. Presence/absence of stonefly genera in the permit area. Oldhouse, Pigeonroost and Seng Camp Creeks will be exposed to operations from the Spruce No. 1 Mine. Frequency of occurrence of particular stonefly taxa across 20 similar mine sites is presented for comparison. NA indicates that the taxon was not collected in Pond et al. (2008) study.

Order	Family	Genus	Oldhouse	Pigeonroost	Seng Camp	Frequency of Occurrence below MTM/VF @ >500 µS/cm*
Plecoptera	Capniidae	*Allocapnia*	X	X	X	0%
Plecoptera	Chloroperlidae	*Alloperla*		X		0%
Plecoptera	Chloroperlidae	*Haploperla*	X			10%
Plecoptera	Chloroperlidae	*Sweltsa*	X			0%
Plecoptera	Leuctridae	*Leuctra*	X	X	X	20%
Plecoptera	Nemouridae	*Amphinemura*	X	X		80%
Plecoptera	Nemouridae	*Ostrocerca*	X	X		0%
Plecoptera	Nemouridae	*Paranemoura*		X		NA
Plecoptera	Peltoperlidae	*Peltoperla*	X		X	10%
Plecoptera	Perlidae	*Acroneuria*	X	X	X	10%
Plecoptera	Perlodidae	*Isoperla*	X		X	25%
Plecoptera	Perlodidae	*Remenus*		X		5%
Plecoptera	Perlodidae	*Yugus*	X			0%
Plecoptera	Pteronarcyidae	*Pteronarcys*	X	X	X	5%
Plecoptera	Taeniopterygidae	*Taenionema*		X		0%
Plecoptera	Taeniopterygidae	*Taeniopteryx*		X	X	5%

Note: *Podmosta, Paraleuctra, Megaleuctra,*and *Beloneuria* reported by Sturm Env. Are likely erroneous identifications[3]. These genera been excluded from this list. *Based on samples from 20 MTM/VF sites Pond et al. (2008).

---

[3] Region III conservatively excluded four stonefly genera from Table 3 which were identified by the permittee's consultant. *Podmosta* is primarily a western U.S. genus, with only one species that occurs in eastern Canada. The Region believes this identification is in error. Also, *Megaleuctra* (while this would be an important, rare sighting) occurs in the eastern part of the state and is not recorded from this ecoregion. Similarly, *Beloneuria* is not known to occur in this ecoregion, but can be confused with the more common *Acroneuria* in early life stages. While *Paraleuctra* can occur in the study area, this genus is most often captured as adults while nymphs are exceedingly rare in benthic samples. We conservatively excluded this genus from the list of stonefly genera found within the Spruce No. 1 project area.

Most of the benthic wildlife taxa naturally occurring in Oldhouse, Pigeonroost and Seng Camp headwaters will not survive in the erosion control ditches proposed for mitigating the loss of headwater streams due to extreme chemical conditions, temperature extremes, and the overall lack of a lotic flow regime found in other examples of these ditches (Kirk 1999, Green et al. 2000, Gingerich 2009). Based on the results shown in Table 1 and 2, some of the most sensitive, but naturally ubiquitous mayfly genera that will likely be extirpated from these streams are:

- Dark Red Quill (*Cinygmula subaequalis*)
- Little Maryatts (*Epeorus* spp.)
- Blue Winged Olives (*Drunella* spp.)
- Sulphurs (*Ephemerella* spp.)
- Blue Quills (*Paraleptohlebia* spp.)
- Brown Duns (*Ameletus* spp.)

Stoneflies predicted to be eradicated from the affected streams (Table 3) include:

- Sallflies: *Alloperla, Haploperla brevis*, and *Sweltsa* spp.
- Salmonfly: *Pteronarcys proteus*
- Browns and Yellows: *Yugus kirchneri*, *Remenus bilobatus*

Populations of other stoneflies such as the Tiny Winter Blacks (*Leuctra* spp.) will be severely reduced. Likewise, populations of other taxa, including caddisflies, will likely be affected. For example, several caddisfly genera present in Spruce No. 1 streams are expected to be affected by authorized project based on their absence from Beech Fork and Left Fork Beech Fork at the Dal-Tex site. The Little Black Caddisfly (*Agapetus*), the Little Black Short-horned Sedge (*Glossosoma*), the Autumn Mottled Sedge (*Neophylax*), the Little Gray Sedge (*Goera*), and Evening Sedge (*Dolophilodes*) have all been extirpated from Dal-Tex streams. Since these data reflect genus-level information, it is unknown whether sensitive species within other genera could also be severely affected or extirpated.

In summary, Pigeonroost, Oldhouse, Seng Camp currently have high taxonomic richness of mayflies and stoneflies. In contrast, many of these taxa have been extirpated in Beech Fork and Left Fork Beech Fork at the Dal-Tex sites (Table 1) indicating the potential for significant effects on these wildlife taxa following the proposed action. Similar patterns of taxonomic loss were observed at 20 other WV sites downstream of MTM/VF when conductivity was greater than 500 µS/cm (Tables 2 and 3), and it is likely that these effects on wildlife taxa and their habitat will occur following the Spruce No. 1 mining operations.

A1.3 Observed/Expected Index: A Measure of Biological Impairment

To supplement multimetric index information (Sec. A1.7), Region III applied another well-accepted and peer reviewed approach, called an Observed/Expected index (O/E) (Hawkins 2006, Van Sickle et al. 2005) (Figure 2) to estimate and quantify the taxa loss from streams impacted from mining activities in the Spruce Fork watershed. This index follows the premise behind RIVPACS (River Invertebrate Prediction and Classification System) after Wright et al. (2000) which models physical or geographic attributes of reference sites in order to predict expected reference taxa.

A1.3.1 Deriving the Observed/Expected Index

O/E measures how far a stream assemblage has departed from reference condition (i.e., biological integrity). O/E ratios represent the number of the expected taxa that are *observed* in a sample, compared to the number *expected* in the sample. Our analysis estimated the number of expected taxa using a set of relatively homogeneous reference sites (i.e., a null model). Setting expectations using a null model is appropriate when working in areas with relatively similar physical and regional characteristics that may have influence on the macroinvertebrate community (e.g., geology, stream slope, natural substrate, season and climate). Because taxa expectations are based on those collected at least-disturbed reference sites, and observations are constrained to those reference site taxa, O/E is an excellent measure of biological integrity. O/E quantifies a fundamental component of ecological capital (Hawkins 2006), one of three general indicators that the National Research Council identified as critical to monitor (NRC 2000).

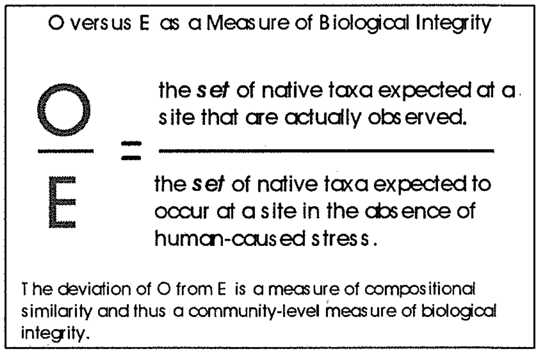


Figure 2. Measure of biological integrity; O vs. E (C.P. Hawkins, Utah State Univ.).

Region III developed two separate O/E models; one model for spring (March-May) and one model for summer (June-Sept). Genus-level null models were developed separately using 128 WVDEP reference sites from ecoregions 67 and 69 (Ridge and Valley and the Central Appalachians, together forming the Mountain Bioregion) sampled in the spring (median catchment area ~1.3 sq. miles), and 181 Mountain Bioregion reference sites sampled in the summer (median catchment area ~2.0 sq. miles). Macroinvertebrate assemblages in these 2 ecoregions are highly similar.

For the WV null models, Region III first calculated the probability of capture (Pc) as the proportion of a taxon's occurrence at all mountain reference sites (combined ecoregions 67 and 69), separately, within spring and summer samples. We did this for all non-chironomid taxa.[4] The Pc's of all taxa with a Pc greater than 0.1 were then summed to

---

[4] The ubiquitous insect family Chironomidae was excluded from null model development since consultants are not currently required by WVDEP or WVDNR to report Chironomidae at the genus level and therefore many do not have expertise in identifying this family at the genus level. Based on probability of capture,

yield the *Expected* number of taxa at a site for the given season (Table 4). Taxa with Pc less than 0.1 were excluded since these taxa are rarely observed (including uncommon sensitive and tolerant taxa, infrequent taxa, or sporadically occurring taxa). Van Sickle et al. (2007) showed increased sensitivity of O/E indices when Pc excluded rarely observed taxa. Using Pc of greater than 0.1 provides a compromise of including most common taxa in the reference taxa pool. Actual taxa collected at test sites (*Observed*), constrained by those reference condition taxa that might be expected, are then compared to what was expected to occur (based on reference condition) and reported as a ratio (O/E).

A site that is a perfect match to the richness of expected indigenous taxa will score 1.0, while downward deviation from 1.0 indicates increasing loss of expected taxa compared to regional reference (e.g., a score of 0.50 indicates a 50% loss of the expected taxa). Upward deviation (greater than 1.0) simply indicates that more taxa were collected than expected. The standard deviation (SD) of reference O/E scores provides a measure of precision; the spring and summer null models both had a SD of 0.19 and represents the upper bound of precision compared to a full predictive model (Van Sickle et al. 2005). These WV null model SDs (0.19) are reasonable, since other full RIVPACS-type models often yield SD's ~0.15. We chose the 5th percentile of reference site O/E scores as an impairment thresholds to correspond to WVDEP's bioassessment threshold for aquatic life use impairment. This O/E 5th percentile was 0.64, indicating that loss of 36% of expected taxa is an unacceptable adverse impact. Van Sickle et al. (2005) cautioned that null models are conservative estimates of impairment and that streams could be misclassified as unimpaired, when in fact, they are impaired. In our models, this conservatism is reflected in the interpretation that more than 1/3 (36%) of the taxa are lost before aquatic life use impairment is detected by the O/E score.

Mayflies+Stoneflies+Caddisflies (EPT taxa) make up a large proportion of the modeled taxa (35 EPT genera for spring, and 36 EPT genera for summer).

Table 4. Probability of capture (Pc) of taxa for WV null model for streams in Mountain Spring and Mountain Summer region of WV. The sum of the Pc is the *Expected* number of taxa used in the O/E index. Only taxa collected greater than 0.10 of sites are included.

Genus	MT Spring	Genus	MT Summer
Leuctra	0.94	Leuctra	0.94
Paraleptophlebia	0.92	Baetis	0.89
Amphinemura	0.89	Paraleptophlebia	0.83
Epeorus	0.88	Dolophilodes	0.83
Ephemerella	0.88	Rhyacophila	0.65
Baetis	0.81	Maccaffertium/Stenonema	0.65
Diplectrona	0.80	Diplectrona	0.64
Rhyacophila	0.75	Hexatoma	0.58
Hexatoma	0.62	Acroneuria	0.57
Haploperla	0.57	Epeorus	0.57
Isoperla	0.57	Ceratopsyche	0.52
Cinygmula	0.55	Cheumatopsyche	0.52
Oulimnius	0.54	Pteronarcys	0.49
Dolophilodes	0.51	Dicranota	0.45
Acroneuria	0.50	Oulimnius	0.45
Maccaffertium/Stenonema	0.50	Simulium	0.45
Sweltsa	0.46	Cambarus	0.43
Polycentropus	0.45	Optioservus	0.41
Cambarus	0.44	Sweltsa	0.41
Pteronarcys	0.43	Polycentropus	0.39
Drunella	0.40	Acentrella	0.38
Dicranota	0.40	Drunella	0.37
Neophylax	0.40	Glossosoma	0.37
Prosimulium	0.38	Nigronia	0.35
Simulium	0.37	Amphinemura	0.32
Lepidostoma	0.37	Ectopria	0.31
Ameletus	0.34	Tallaperla	0.31
Yugus	0.34	Leucrocuta	0.30
Cheumatopsyche	0.31	Psephenus	0.28
Antocha	0.26	Ephemerella	0.26
Ceratopsyche	0.25	Hydropsyche	0.26
Bezzia/Palpomyia	0.25	Isonychia	0.25
Acentrella	0.23	Isoperla	0.24
Optioservus	0.23	Diphetor	0.23
Wormaldia	0.22	Gammarus	0.23
Tipula	0.22	Peltoperla	0.22
Leucrocuta	0.20	Antocha	0.22
Peltoperla	0.20	Lepidostoma	0.21
Diphetor	0.19	Yugus	0.19
Gammarus	0.19	Bezzia/Palpomyia	0.18
Nigronia	0.19	Plauditus	0.18
Ectopria	0.18	Stenacron	0.17
Hydropsyche	0.18	Tipula	0.17
Stenacron	0.15	Haploperla	0.16
Ephemera	0.14	Dixa	0.15
Pseudolimnophila	0.14	Stenelmis	0.15
Chelifera	0.13	Heptagenia	0.14
Isonychia	0.13	Ephemera	0.13
Blephariceraa	0.13	Malirekus	0.12
Tallaperla	0.12	Eurylophella	0.12
Pycnopsyche	0.11	Wormaldia	0.11
Sum of Probability of Capture	20.37	Sum of Probability of Capture	18.72

A1.3.2 O/E Comparisons in Spruce No. 1 streams vs. Dal-Tex streams

Region III compared benthic assemblages in streams at the Spruce No. 1 project area (Pigeonroost, Oldhouse, and White Oak) to streams at adjacent mining operations including Dal-Tex (L.F. Beech, and Beech) and Rockhouse Creek using the WV null model of O/E (after Van Sickle et al. 2005).[5] The O/E index results indicate that streams with adjacent fills receiving drainage from MTM/VF operations and the macroinvertebrate communities within those streams are consistently degraded (Tables 5 and 6), based upon the O/E threshold value of 0.64.

Table 5. Null model O/E scores for EPA and WVDEP data for Spruce No. 1 project area and immediately adjacent valley filled streams.

EPA Data-Spring

Station ID	Type	Stream Name	Date	Observed	Expected	O/E
KC-00046-1.1	Adjacent Fills	Rockhouse Creek	4/21/1999	7	20.37	0.34
KC-00046-1.1	Adjacent Fills	Rockhouse Creek	4/25/2000	8	20.37	0.39
KC-00046-1.1	Adjacent Fills	Rockhouse Creek (Replicate)	4/25/2000	4	20.37	0.20
KC-00047-2	Adjacent Fills	Beech Creek	4/21/1999	6	20.37	0.29
KC-00047-2	Adjacent Fills	Beech Creek	4/27/2000	5	20.37	0.25
KC-00047-2	Adjacent Fills	Beech Creek (Replicate)	4/27/2000	4	20.37	0.20
KC-00049-0.2	Adjacent Fills	Left Fork/Beech Creek	4/21/1999	7	20.37	0.34
KC-00049-0.2	Adjacent Fills	Left Fork/Beech Creek	4/25/2000	4	20.37	0.20
KC-00054-0.9	Spruce No. 1	Pigeonroost Branch	4/22/1999	24	20.37	1.18
KC-00054-0.9	Spruce No. 1	Pigeonroost Branch	4/25/2000	14	20.37	0.69
KC-00055-0.4	Spruce No. 1	Oldhouse Branch	4/22/1999	19	20.37	0.93
KC-00055-0.4	Spruce No. 1	Oldhouse Branch	4/25/2000	14	20.37	0.69
OECA 14	Spruce No. 1	Oldhouse Branch	3/14/2007	24	20.37	1.18
KC-00061-0.4	Spruce No. 1	White Oak Branch	4/22/1999	21	20.37	1.03
KC-00061-0.4	Spruce No. 1	White Oak Branch (Replicate)	4/22/1999	24	20.37	1.18
KC-00061-0.4	Spruce No. 1	White Oak Branch	4/25/2000	16	20.37	0.79
OECA 13	Spruce No. 1	White Oak Branch	3/14/2007	23	20.37	1.13

EPA Data-Summer

Station ID	Type	Stream Name	Date	Observed	Expected	O/E
KC-00046-1.1	Adjacent Fills	Rockhouse Creek	7/29/1999	6	18.72	0.32
KC-00047-2	Adjacent Fills	Beech Creek	7/29/1999	7	18.72	0.37
KC-00049-0.2	Adjacent Fills	Left Fork/Beech Creek	7/29/1999	5	18.72	0.27
KC-00055-0.4	Spruce No. 1	Oldhouse Branch	7/29/1999	14	18.72	0.75
KC-00054-0.9	Spruce No. 1	Pigeonroost Branch	7/29/1999	18	18.72	0.96

WVDEP Data-Summer

Station ID	Type	Stream Name	Date	Observed	Expected	O/E
KC-00046-0	Adjacent Fills	Rockhouse Creek	8/6/2002	7	18.72	0.37
KC-00046-0.8	Adjacent Fills	Rockhouse Creek	8/6/2002	9	18.72	0.48
KC-00047-0	Adjacent Fills	Beech Creek	8/6/2002	10	18.72	0.53
KC-00047-0	Adjacent Fills	Beech Creek	8/6/2002	7	18.72	0.37
KC-00047-1.7	Adjacent Fills	Beech Creek	8/7/2002	10	18.72	0.53
KC-00049-0.2	Adjacent Fills	Left Fork/Beech Creek	8/7/2002	5	18.72	0.26
KC-00054-0.8	Spruce No. 1	Pigeonroost Branch	8/6/2002	13	18.72	0.69
KC-00061-0.5	Spruce No. 1	White Oak Branch	7/10/2000	14	18.72	0.75

---

[5] Note that Seng Camp Creek was not included in this analysis because we did not have a sample collected with quantitatively comparable methods.

Table 6. Summary of WV O/E null model results for the Spruce No. 1 Project area (EPA data). The biological impairment threshold is 0.64 (corresponding to the 5th percentile of WVDEP reference site distributions). An O/E score of ~1.0 means that the number of Observed native taxa is equivalent to the Expected number of native taxa.

Table 6. Summary of WV O/E null model results for Spruce No. 1 Project			
		Mean (SD) O/E	
	Spruce No. 1	**Dal-Tex**	
	Pigeonroost,Oldhouse, White Oak	Beech, LF Beech	Rockhouse
Spring	0.98 (0.20); n=9	0.26 (0.06);n=5	0.31 (0.10); n=3
Summer	0.85 (0.15); n=2	0.32 (0.08); n=2	0.38 (0.08); n=2
• Adjacent mined sites include LF Beech, Beech, and Rockhouse			
• The highest O/E scores were recorded in Pigeonroost, Oldhouse, and White Oak (each scored 1.18)			
• The lowest O/E scores were recorded in Beech and LF Beech on Dal-Tex (each scored 0.20)			
Based on WVDEP Mountain reference sites, on average:			
• Spruce No. 1 samples are missing ~2% of expected taxa in Spring, and ~15% in Summer			
• Dal-Tex sites are missing ~74% of expected taxa in Spring, and ~68% in Summer.[6]			
• SD for Spruce No. 1 streams had similar or better precision (SD) to the WVDEP reference model			
• SD for Dal-Tex was very low indicating that all observations consistently show missing taxa			

The model indicates that Spruce No. 1 sites are comparable to unimpaired WVDEP mountain ecoregion reference sites, and past mining by Mingo Logan Coal Co. has led to the estimated extirpation of ~70% of the native expected taxa in streams draining their adjacent Dal-Tex mine operation. This finding is significant, and supports the conclusion that conditions in upper Seng Camp, Pigeonroost, and Oldhouse will follow this pattern of genus-level extirpation and aquatic life use impairment if the project commences as proposed.

A1.3.3 O/E Relationships to Stressors

Using the WV spring null model applied to MTM genus-level data from Pond et al. (2008), O/E scores were negatively correlated ($r^2 = 0.63$) to conductivity which explained more than twice the variance than RBP habitat scores ($r^2 = 0.28$)), confirming that conductivity is an excellent predictor of native taxa loss from Appalachian streams (Figures 3 and 4). All sites in Pond et al. (2008) were located upstream of any residences, and thus MTM was the sole or dominant landuse. The majority (85%) of mined sites assessed in Pond et al. (2008) having conductivity greater than 500 μS/cm,

---

[6] Based on EPA data (Pond et al. 2008), all mined sites lost 47% of expected taxa, on average.

were impaired (based upon an impairment threshold equal to the 5th percentile of the 128 WVDEP spring reference sites and a value roughly equal to 1.96 SD of reference O/E).

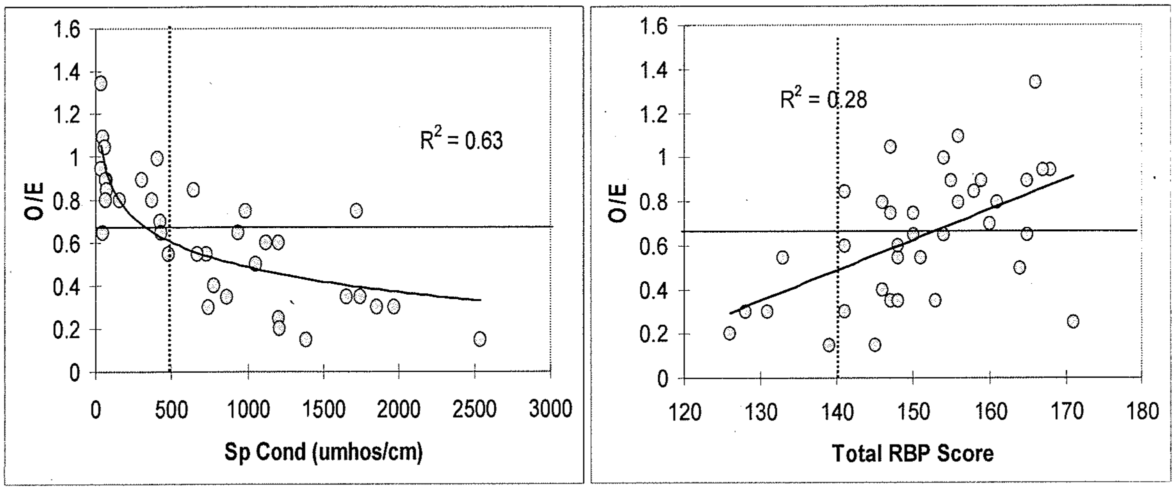


Figure 3. Relationship between Spring O/E null model, specific conductance (logarithmic fit), and RBP Habitat scores (linear fit) applied to data from Pond et al. (2008). Vertical dashed lines indicate 500 µS/cm threshold and 140 RBP score threshold for WVDEP reference screening criteria. Solid horizontal lines represent the 5th percentile of 128 WVDEP Mountain reference sites (the impairment threshold).

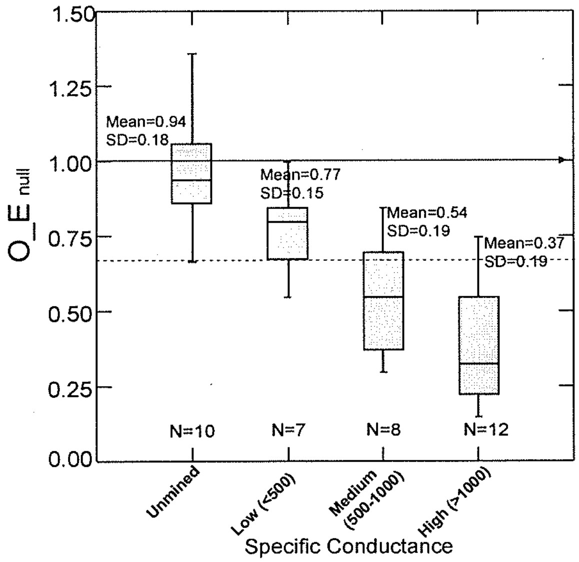


Figure 4. Boxplot of O/E scores along generalized specific conductance categories. Data are from Pond et al. (2008). Dashed line indicates 5th percentile impairment threshold calculated from 128 WVDEP independent reference sites.

In an effort to explore the actual contributions of abiotic stressors to O/E score variation using raw MTM/VF data from Pond et al. (2008), a multiple regression analysis was performed on several habitat metrics plus conductivity, temperature, and pH across the 37-site dataset. Three separate regression models were run (Table 7). O/E scores were normally distributed but logarithmic transformations were applied to abiotic parameters to satisfy assumptions of normality. The analysis shows that conductivity was the only metric significantly correlated with O/E values. Regression coefficients were not significant for sediment deposition, substrate embeddedness, channel alteration, riparian zone width, pH, or temperature. In fact, habitat offered little explanatory value in O/E variation (Table 7). While Region III acknowledges habitat can strongly affect native wildlife assemblages, these additional analyses support previous results (Pond et al. 2008) that the variable water quality gradient (measured as conductivity) across MTM and un-mined watersheds strongly structured wildlife communities (O/E). It is likely that similar water chemistry induced patterns of taxa loss will occur downstream of Spruce No. 1 following the proposed action.

Table 7. Multiple regression statistics for O/E scores and abiotic variables (Full Model, Sp. Cond Model, and Sp. Cond+RBP Habitat Model).

Full model		log Sp. Cond		log Sp Cond + logHabitat	
Regression Statistics		*Regression Statistics*		*Regression Statistics*	
Multiple R	0.84	Multiple R	0.79	Multiple R	0.80
R Square	0.71	R Square	0.63	R Square	0.65
Adjusted R Square	0.63	Adjusted R Square	0.61	Adjusted R Square	0.62
Standard Error	0.18	Standard Error	0.18	Standard Error	0.18
Observations	37	Observations	37	Observations	37

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	7	2.1805	0.3115	9.9263	0.0000
Residual	29	0.9100	0.0314		
Total	36	3.0905			

Full model	*Coefficients*	*St. Error*	*t Stat*	*P-value*
Intercept	0.23	0.82	0.28	0.78
log Cond	-0.29	0.07	-4.34	0.00
logEmbed	0.14	0.26	0.56	0.58
logChanAlt	0.59	0.51	1.15	0.26
logSedDep	0.45	0.27	1.65	0.11
logRiparian	-0.07	0.33	-0.23	0.82
logTemp	0.26	0.33	0.79	0.44
pH	-0.05	0.05	-0.97	0.34

A1.4 Bioassessments Using WVDEP's WVSCI

States routinely use macroinvertebrate assemblage data to assess their narrative water quality standards and to determine support of aquatic life uses. WVDEP uses a family-level multi metric index called the WV Stream Condition Index or WVSCI.
The WVSCI uses six (6) component metrics to summarize and analyze family-level macroinvertebrate taxa lists. The six metrics are total number of EPT (Ephemeroptera, Plecoptera and Trichoptera or mayflies, stoneflies and caddisflies) taxa, total number of taxa, % of organisms that are EPT, % of organisms that are Chironomidae (midges), the % of organisms in the top two dominant taxa, and the Hilsenhoff Biotic Index. All metrics are computed at the family-level with a 200 fixed count subsample. The metrics are scored against Best Standard Values (BSVs) for the entire dataset, as a % of the BSV and normalized to a score of 100. The average of all six metrics makes up the final WVSCI score. The lower the score, the more degraded the macroinvertebrate assemblage. For more information on the WVSCI, go to http://www.wvdep.org/Docs/536_WV-Index.pdf.

Currently, WVDEP uses WVSCI index scores greater than 68 to indicate full support of the aquatic life use. This score represents the lower 5th percentile of the range of scores of the 107 reference site scores used in the 2000 report (Gerritsen et al. 2000). WVDEP has not updated the WVSCI threshold with new reference site data collected since the WVSCI report was published in 2000. Since that time, WVDEP has sampled hundreds of new reference sites. There are now 394 statewide reference sites available in the WVDEP dataset. If all reference sites are included in the statewide reference condition, the 5th percentile of the reference scores increases to 72.

EPA sampled several streams within the Spruce Fork watershed for the Mountaintop Mining/Valley Fill Programmatic Environmental Impact Study (MTM/VF PEIS) (Green et al. 2000; Bryant et al. 2002). These data indicate that the unmined streams within and near the project area, including White Oak Branch, Oldhouse Branch, and Pigeonroost Branch, were high quality streams that fully support the aquatic life use, based on the family-level WVSCI (Table 8) and water quality (Table 9) data. The streams located in the historically MTM/VF mined areas located nearby (Rockhouse Branch, Beech Creek, and the Left Fork of Beech Creek) were degraded. These data indicate that the aquatic life use in streams on the project area (i.e., Oldhouse Branch and Pigeonroost Branch) will likely be degraded to the conditions exhibited in the Beech Creek and Rockhouse sub-watersheds after they are mined.

Table 8. WVSCI scores for EPA sites on and near the proposed project area. (USEPA MTM PEIS Study Green et al. 2000).

Stream Classification	Stream Name	Spring 1999	Summer 1999	Fall 1999	Winter 2000	Spring 2000
Unmined	White Oak Branch	87	**	**	67.8	90.2
Unmined	Oldhouse Branch	94.9	78.6	**	91.4	90.2
Mined*	Pigeonroost Branch	86.5	80.8	88.8	94.2	82.6
Filled	Rockhouse Creek	48.2	54.7	69.4	50.6	51.6
Filled	Beech Creek	55.9	48.6	58.3	48.7	48.6
Filled	Left Fork of Beech Creek	56.4	59.8	**	**	37.6

* Pigeonroost Branch has some limited mining (no valley fills) in the headwaters region of the watershed.

**Drought/ Low Flow Conditions - Unable to Collect a Representative Sample

Table 9 indicates average values for selected water quality parameters for the benthic macroinvertebrate sites on streams within the proposed project area and for the nearby mined streams. These data indicate water quality degradation at the mined sites compared to the unmined sites. The PEIS study found that the changes in macroinvertebrate assemblages and WVSCI scores were strongly correlated to water quality changes. Again, these data indicate that the water quality in streams on the proposed mine area will likely be degraded in a similar fashion, and will likely result in degradation of the aquatic life use in the mined tributaries. The proposed project will also contribute increased loadings of pollutants to downstream waters in Spruce Fork. See Appendix 2 for further detailed findings on water chemistry impacts.

Table 9. Average water quality at EPA sites on and near the proposed project area: August 2000 to February 2001 (USEPA MTM PEIS Study Bryant et al. 2002)

Site Description (Category)	Alkalinity (mg/L)	Calcium (mg/L)	Sulfate (mg/l)	Tot. Dissolved Solids (mg/L)
White Oak Branch (Unmined)	34.7	11.8	21.6	78.7
Oldhouse Branch (Unmined)	21.7	9.6	18.1	55.7
Rockhouse Creek (Fills)	255	108.1	415.8	848.7
Beech Creek (Fills)	172.8	84.9	350.7	679.3
Left Fork Beech Creek (Fills)	501.8	269	1110.7	2071.7
Range of Increase (Mined / Unmined)	5x to 23x	7x to 28x	16x to 61x	8x to 37x

Table 10 shows WVSCI scores for macroinvertebrate data collected by WVDEP at a subset of sites in the Spruce Fork, Pond Fork, and Little Coal watersheds. Most of these data were collected in the summer of 2002, but a few samples were collected in the fall of 1997, the spring of 2005 and the spring of 2008.

Table 10. WVSCI scores for selected WVDEP sites on Spruce Fork, Pond Fork and Little Coal River. NA= no biological sample taken but water chemistry data from these sites are reported in Appendix 2.

Table 10. WVSCI scores for selected WVDEP sites on Spruce Fork, Pond Fork and Little Coal River.

Stream Name (mile point)	WVSCI	Fully Supports Aquatic Life Use using WVSCI > 72
Tribs to Spruce Fork draining Spruce No.1- unmined[7]		
Seng Camp Creek (at mouth)	73.8	X
Pigeonroost Branch (mile 0.8)	62.9	
Oldhouse Branch (at mouth)	NA	
White Oak Branch (mile 0.5)	86.5	X
Tribs to Spruce Fork draining nearby mined areas		
Rockhouse Creek (mile 0.8)	52.9	
Beech Creek (at mouth)	58.8	
Left Fork Beech Creek	23.4	
Trace Branch	NA	
Spruce Fork mainstem sites		
Spruce Fork (mile 0.3)	73.1	X
Spruce Fork (mile 0.5)	69.3	
Spruce Fork (mile 4.6)	72.5	X
Spruce Fork (mile 6)	61.4	
Spruce Fork (mile 9.6)	41.4	
Spruce Fork (mile 11.4)	65.0	
Spruce Fork (mile 14.4)	69.5	
Spruce Fork (mile 17.2)	67.9	
Spruce Fork (mile 18.1)	67.1	
Spruce Fork (mile 18.5)	53.8	
Spruce Fork (mile 18.6)	NA	
Spruce Fork (mile 23.7)	69.0	
Pond Fork mainstem sites		
Pond Fork (mile 0.3)	68.1	
Pond Fork (mile 0.4)	73.1	X
Pond Fork (mile 4.9)	64.3	
Pond Fork (mile 6.3)	64.7	
Pond Fork mile 9.0)	66.8	
Pond Fork (mile 12.6)	NA	
Pond Fork (mile 15.8)	64.1	
Pond Fork (mile 21.6)	61.3	
Pond Fork (mile 24.4)	59.1	
Pond Fork (mile 26.6)	72.2	X
Pond Fork (mile 32.3)	57.5	
Little Coal mainstem sites		
Little Coal River (mile 0.2)	NA	
Little Coal River (mile 3.6)	73.9	X
Little Coal River (mile 4.7)	NA	
Little Coal River (mile 10.2)	NA	
Little Coal River (mile 16.5)	NA	

---

[7] Seng Camp Branch is approx. at Spruce Fork RM 17.5
Pigeonroost Branch is approx. at Spruce Fork RM 20.8
Oldhouse Branch is approx. at Spruce Fork RM 21.5
White Oak Branch is approx. at Spruce Fork RM 24.6

Table 10. continued

Stream Name (mile point)	WVSCI	Fully Supports Aquatic Life Use using WVSCI > 72
Little Coal River (mile 17)	65.2	
Little Coal River (mile 17.2)	64.5	
Little Coal River (mile 17.8)	NA	
Little Coal River (mile 21.7)	66.8	
Little Coal River (mile 25.2)	NA	

The WVDEP data indicate that streams on the nearby Dal-Tex operation do not fully support the aquatic life use, based on the WVSCI scores. This impairment is due to mining in the tributaries draining the Dal-Tex mine. Note that the WVDEP data indicate a lower WVSCI scores for Pigeonroost Branch than the scores in the EPA dataset. This lower score is likely because the WVDEP sample was taken further down the watershed, and downstream of a house where immediate riparian and channel impacts were obvious.

Overall, these WVDEP data support the EPA data (Green et al. 2000; Bryant et al. 2002) and the finding that the aquatic life use is impaired in nearby mined streams and on the mainstem of Spruce Fork, Pond Fork and the Little Coal River. The impairment in the mainstem of Spruce Fork, Pond Fork, and the Little Coal are likely due to a combination of stressors, including mining and residential stressors.

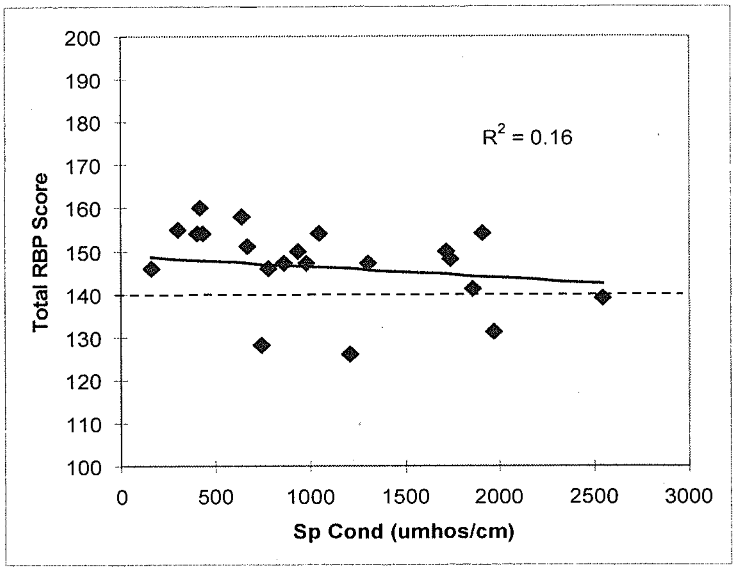
Based on the foregoing, Spruce Fork has little, if any, capacity to assimilate additional pollutants from the proposed project. The proposed project would further degrade the waters of Spruce Fork.

A1.5 Comparison of Biological Responses to Impoundments versus Mining

The construction and operation of in-stream mining impoundments can also affect downstream communities, notably by altering natural food sources, temperature regimes, and flow regimes. Using data collected from the PEIS in WV, Armstead et al. (2004) found that the "most significant changes in stream biological community are the shifts in the functional feeding groups toward more filter feeding organisms and the reduction of the mayfly community in [downstream of] [valley]fill and fill/residentially influenced sites." Their analysis showed that scrapers (algal grazers) and shredders (feed upon fallen leaves) (most of these functional feeders are mayflies and stoneflies) were strongly reduced below valleyfills in the winter and spring, respectively. Armstead et al. (2004) believed that "the changes in community structure may result from the presence of ponds and changes in temperature regimes." They further stated that "the reduced mayfly populations in the fill and fill/residentially influenced sites are not uncommon in areas with mining influence or below impoundments."

While impacts to aquatic communities can occur directly from instream impoundments, it does not appear from the data that impoundments are the primary causes of aquatic life impairment below valley fills. Filter feeders generally increase below impoundments, but long after mine ponds have been removed, chemically tolerant filter feeding caddisflies persist and dominate the samples. In contrast to Armstead et al.'s claim that the presence of mining sediment ponds causes the impact to aquatic wildlife via alteration of the hydrological regime and changes in food resources, EPA Region III data show that communities below all mining sediment control ponds (n=21) respond strongly to a specific conductance gradient. Additionally, EPA established that there was a poor correlation between total habitat score and conductivity (Figure 5; data from Pond et al. 2008) at the impounded mine sites (n=21). In the 500 to 1000 µS/cm range, 6 out of 7 sites had good habitat quality (greater than 140; based on WVDEP reference screening criteria). In the 1000 to 2500 µS/cm range, 7 out of 9 sites had acceptable total habitat scores. These results indicate that habitat was not limiting at the majority of the mined sites sampled by EPA.

Figure 5. Relationship between RBP habitat score and specific conductance obtained from receiving streams of mine site impoundments . Data from Pond et al. (2008). Dashed line demarcates criterion required for WVDEP reference site candidacy.



Even when sediment ponds were removed as a variable by examining only sites below ponds, conductivity explained 80% of the variance associated with mayfly generic richness (Figure 6). Region III found that when ponds had been removed during reclamation, degraded communities persisted and were dominated by tolerant organisms, indicating that the impoundment was not the main reason for impaired biological communities. Significant degradation of water quality is much more strongly correlated to the adverse impact on macroinvertebrate community structure below MTM operations with valleyfills such as that proposed by the Spruce No. 1 mine than the presence of sediment ponds.

Impounded Sites Only

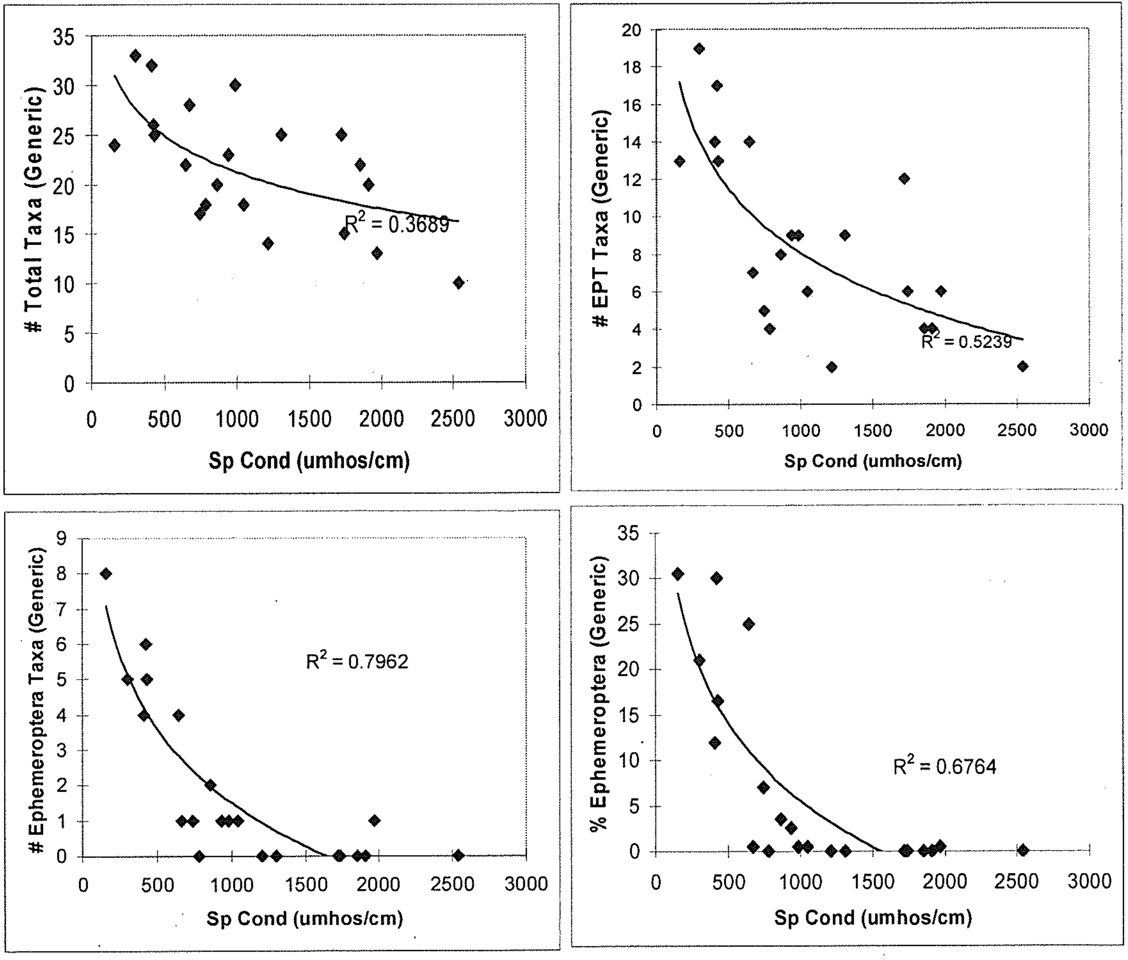


Figure 6. Relationships between conductivity and macroinvertebrate metrics (Total Generic Richness, EPT Generic Richness, Ephemeroptera Generic Richness, and % Ephemeroptera Abundance) for mine site receiving streams located below in-stream impoundments (EPA R3 data). These sites have no residential development upstream of the sample site. If the macroinvertebrate responses were caused solely by the presence of up-stream sediment ponds, the relationship with conductivity would be insignificant. Instead, the loss of taxa is strongly and significantly related to conductivity.

Armstead et al. (2004) concluded that impoundments, not degradation of water quality, caused the shift in functional feeding groups downstream of valley fills. Similar to Armstead et al. (2004), Region III found that functional feeding groups (based on WVDEP group designations) also shift in relation to mining impacts. However, Region III believes the shift is more related to water quality. Table 11 shows that % Scrapers (herbivorous grazers) was significantly reduced, while filter-feeding collectors significantly increased. Moreover, these two feeding groups responded strongly to conductivity (data from Pond et al. 2008). This analysis confirms that not only was community structure affected, but functional feeding group composition was significantly altered below MTM/VF operations.

Table 11. Mean relative abundance of functional feeding groups at unmined and mined sites (data from Pond et al. 2008). Mann-Whitney U-tests shown. Spearman correlation to specific conductance is also provided.

Mann-Whitney U-Test				Spearman Correlation Matrix	
FFG	Unmined	Mined	*p*-value		Sp. Cond.
% Collector-Gatherers	29.9	32.4	0.76	% Collector-Gatherers	0.01
% Scrapers	28.2	5.3	<0.0001	% Scrapers	-0.79
% Collector-Filterers	8.0	27.2	0.003	% Collector-Filterers	0.62
% Predators	8.6	6.6	0.12	% Predators	-0.41
% Shredders	25.0	28.2	0.72	% Shredders	-0.22
Other	0.2	0.3	0.85	Other	0.01

To test whether collector-filterers are solely related to impoundment as proposed by Armstead et al. (2004, 2006), we compared the relative abundances at EPA sampled mined sites below sediment ponds along the conductivity gradient. As in the above analysis of community metrics, Figure 7 shows that despite impoundments being present at all sites, the % Filter Feeders (collector-filterers) metric showed a strong response to conductivity. Low collector-filterer abundance was present at the more dilute sites, and increased with rising conductivity. At these 21 sites, conductivity was not significantly related to distance below impoundments (ranging from 0.05 to 1.63 km; r^2=0.14, p=0.98)[8]. This provides evidence that water quality has a greater effect on macroinvertebrate communities than habitat changes from impoundments below MTM/VF. Further, these water quality changes can affect trophic (feeding) group composition.

---

[8] However, at <u>all</u> 27 mined sites used in Pond et al. (2008), conductivity was weak but significantly related to distance from valleyfills (r^2=0.23, p=0.009).

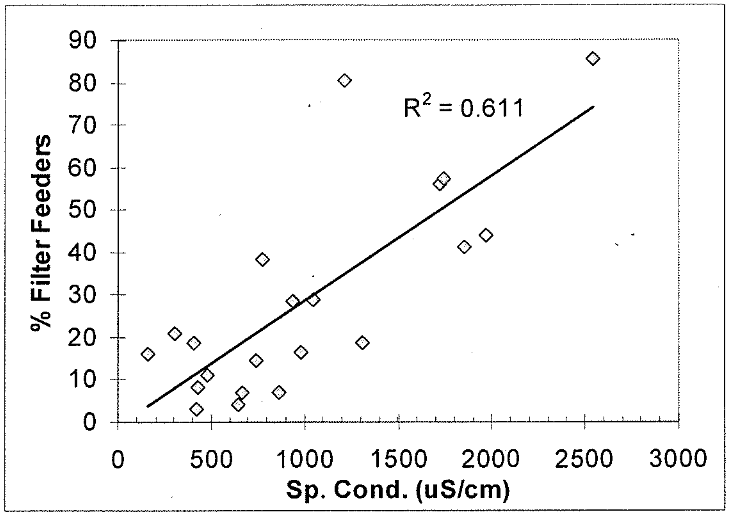


Figure 7. Relationship between relative abundance of collector-filterers (% Filter Feeders) and conductivity for only those mine site receiving streams located below in-stream impoundments (EPA R3 data).

A1.6 Appropriateness of data sets and analyses

In order to be used as a basis for quantitative scientific analyses, datasets must conform to a minimum standard of quality, where the sampling design and data analysis techniques are both appropriate and properly implemented, and results are appropriately interpreted. Previous reports presented by the Corps utilized datasets that inappropriately collected and analyzed data, and as such, should not be considered reliable. In the Corps' evaluation of comments submitted by EPA for the Spruce mine (USACE Memorandum for the Record dated September 30, 2009), the Huntington District cited three studies they believe contradict the conclusions of Pond et al. (2008).[9] One of these studies (Hartman et al. 2005), actually shows strong significant effects on macroinvertebrates, contrary to the USACE interpretation of the results. With respect to the other two studies, EPA has reviewed actual or similar industry datasets that Armstead et al. and the USACE relied upon, and has found that the majority of the data are of poor quality, with low taxonomic precision and quality assurance, and with many errors in metric and index calculations. Sample sites are often pseudo-replicated and effects of natural variation such as seasonality and stream size are uncontrolled. Samples are often collected out of the April-October index period and the WVSCI is used inappropriately. Therefore we believe the conclusions in the Armstead et al. (2006) report and the USACE memorandum are unreliable.

---

[9] Unlike Pond, et al (2008), to EPA's knowledge these studies had neither been peer reviewed nor published.

A1.6.1 Aggregate %EPT Abundance Metric vs. EPT Generic Richness and Components

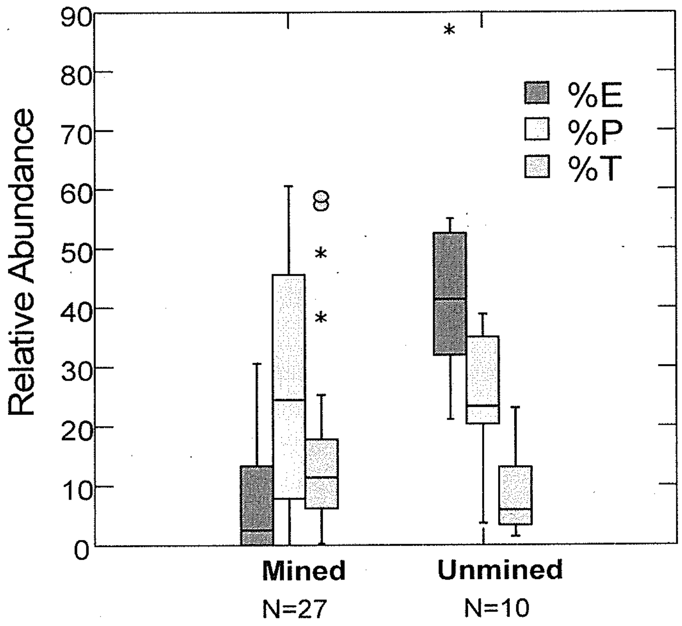
EPA uses Ephemeroptera richness to detect impact from mining instead of % EPT abundance metric because the commonly used %EPT (relative abundance of mayflies+stoneflies+caddisflies) metric can often be one of the most misleading benthic metrics used in headwater streams of the Appalachian coalfields. It is misleading because relative abundance metrics can mask shifts in species composition if there are increases in dominant taxa while sensitive taxa decline. Specifically some of the tolerant, component genera of EPT increase in abundance following disturbance and dominate streams influenced by chemical or habitat stress, while the more sensitive members of these orders decline or are extirpated. Hence, while the %EPT metric indicates that a high percentage of the individuals in the sample belong to those orders, it does not reveal that the more sensitive *genera* have been extirpated and the remaining individuals at mined sites are largely tolerant or facultative members of those orders. EPA R3 found that two components of %EPT (%P and %T) showed no significant difference between Unmined and MTM streams in WV due to the presence of tolerant genera within those generally sensitive orders. It is important to note that in streams with discharges of severely degraded water (e.g., conductivity indicator greater than 2000 μS/cm), even the more tolerant species can be harmed.

In headwater streams(e.g., less than 3 sq. miles), %EPT is not an effective indicator of impairment because several pollution tolerant taxa belonging to the orders Ephemeroptera (e.g., *Baetis, Plauditus, Caenis, Isonychia*), Plecoptera (e.g., *Amphinemura, Allocapnia, Taeniopteryx*) and Trichoptera (mostly Hydropsychidae, Hydroptilidae, and the philopotamid, *Chimarra*) are typically not present or naturally abundant in these streams. These taxa have life history strategies and physiological adaptations that allow them to become dominant in streams under increasing chemical or habitat stress. The occurrence of these tolerant taxa within the generally sensitive EPT orders, results in a metric that can not consistently distinguish effects due to environmental stressors. To demonstrate this, Figure 8 shows the distribution of the three component orders of %EPT across mined and unmined streams from Pond et al. (2008). Mann-Whitney U-tests (SYSTAT v. 13) were performed to determine differences in mean relative abundance of %E, %P, and %T between the two categories (Mined and Unmined). There was no significant difference between mined and unmined sites for both %P and %T. On average, %T was greater at mined sites and was attributed to increases in tolerant hydropsychids, hydroptilids, and/or philopotamids. % P was often dominated at mined sites by the facultative nemourid, *Amphinemura,* while many sensitive stoneflies typical of unmined sites were extirpated. This is such a commonly recognized issue that some states (e.g., PA and VA) have excluded more tolerant and ubiquitous genera or families such as *Baetis* and Hydropsychidae before calculating %EPT.

For example, hydropsychid caddisflies can account for greater than 90% of the total sample abundance and is a common biological signature in streams draining some MTM/VF operations. This dominance indicates severe water quality degradation, but these caddisflies can tolerate these degraded conditions, even when coated in oxides of

Mn and Fe precipitate, and calcium minerals as shown in Figure 9. In this unnamed tributary to Leatherwood Creek (Clay Co., WV), %EPT was 99% and the WVSCI score was 47.4, despite only having 4 total genera in the 200 organism subsample. Clearly, in this example, the %EPT metric is providing a false positive signal, indicating a high proportion of the sample is composed of nominally sensitive EPT individuals, while in fact only a single tolerant caddisfly is present, and most taxa were extirpated at the site. While this WVSCI scores is low enough to indicate impairment, other scores can be high enough for %EPT to obscure any relationships (i.e., correlations and regression-type analyses) between biological response metrics and stressors.

The inability of this metric to detect wholesale changes in the invertebrate community from a diverse, sensitive assemblage to one dominated by tolerant individuals of a few taxa makes it an inappropriate metric to elucidate water quality effects on macroinvertebrate communities. Further, a reliance on %EPT can lead to misinterpretation of biological condition or stressor-relationships. Because genus-level taxonomic data and genus-based community metrics allow for the detection of shifts in community composition, they are a more accurate way of assessing impacts and should be used, where available, and limitations of the accuracy and variability of certain metrics (e.g. %EPT) must be understood.



	WVDEP REF (n=128)	Unmined (n=10)	Mined (n=27)	Mann-Whitney U-test (p-value)
% E	36.1	44.1	7.4	5.5 (p<0.000)
% P	23.0	24.3	27.4	144.0 (p=0.76)
% T	11.4	8.5	16.2	174.0 (p=0.18)

Figure 8: Boxplot comparison of relative abundance of EPT component insect orders across Mined and Unmined sites (EPA data from Pond et al. 2008). Mean values (including WVDEP Reference sites (Mountain Spring bioregion) and Mann-Whitney U-tests results included (table insert). See Figure 10 for comparison of E, P, and T richness. Only %E is significantly different.

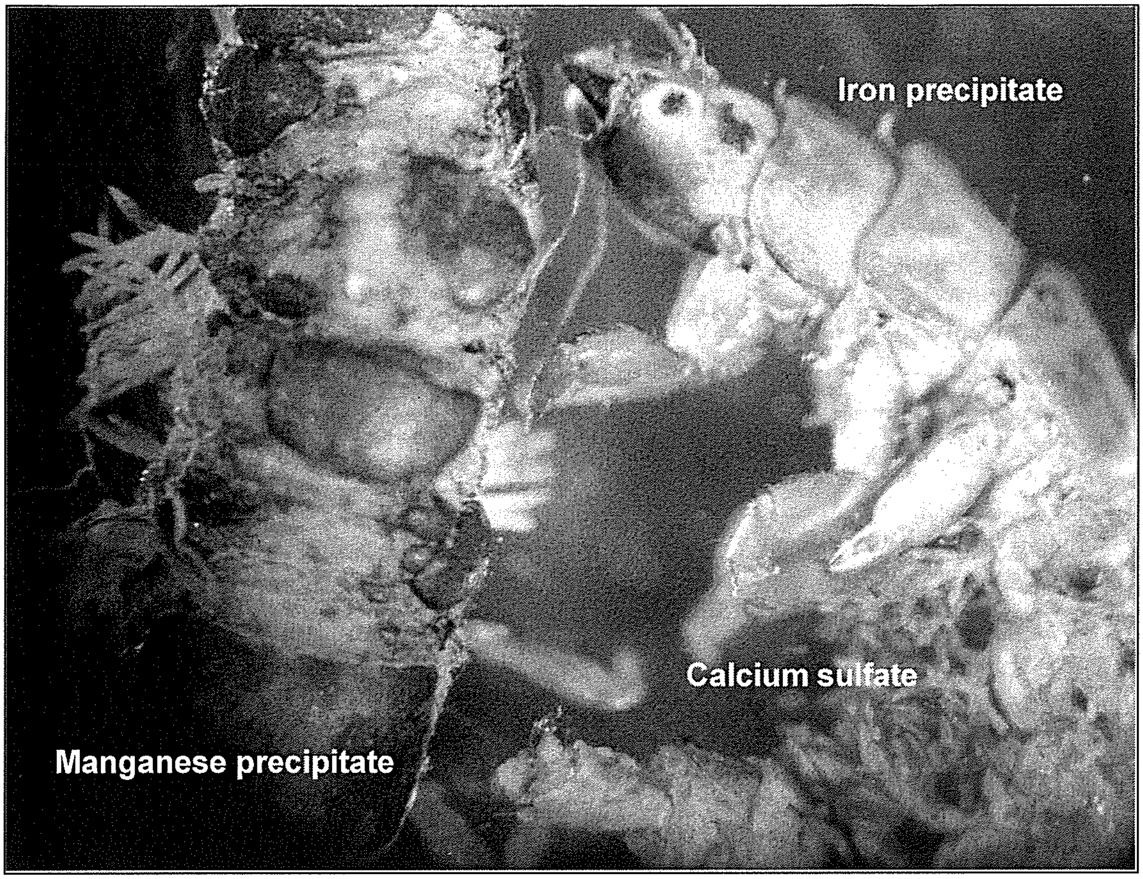
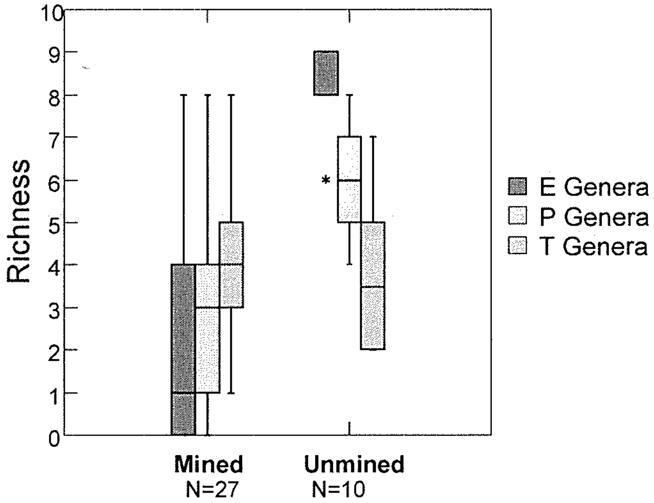


Figure 9. Evidence that %EPT can be an unreliable metric. In the entire sample (UT Leatherwood Creek, Clay Co., WV), the hydropsychids, *Hydropsyche* and *Cheumatopsyche* (shown), comprised over 99% of all organisms. The animals are tolerant of poor water quality resulting in heavy coatings of oxides of Mn, Fe, and possibly Ca-carbonate-sulfate based minerals. On the left, black manganese oxide concretions are shown in several locations on the insect's body surface. On the right, the normally thin white, filamentous gills of this hydropsychid are heavily thickened by mineral precipitates.

A1.6.2 EPT Genus Richness is a Better Indicator to Protect Native Aquatic Diversity

EPT genus-level richness is a much more accurate and sensitive metric than %EPT, as it can detect shifts in community composition, rather than just the relative abundance of one to a few tolerant genera (e.g., *Baetis*, *Amphinemura*, *Cheumatopsyche*). While EPT richness is a much more appropriate measure in these streams, it can still, on occasion, give false-positives of acceptable stream quality because it aggregates across multiple taxa with varying responses to disturbance. For example, caddisfly richness was not significantly sensitive to mining influence in Pond et al. (2008), whereas stonefly and mayfly richness was significantly altered at mined sites (Figure 10). So, by using EPT as an aggregate metric, a false positive assessment could occur when the diversity of mayflies and stoneflies are affected, but not caddisflies.

In the case of the Spruce No. 1 streams, however, caddisflies were relatively diverse (14 total genera) compared with streams draining Dal-Tex (7 total genera), which indicates that the effects of mining in these systems can be detected using this aggregate metric. Conditions in mined streams near Spruce No. 1 exhibit significant reductions in E, P, and T taxa richness (Figure 11). These data indicate that the proposed project is likely to cause similar impacts.



	WVDEP REF (n=128)	EPA Unmined (n=10)	EPA Mined (n=27)	Mann-Whitney U test (p-value)
E Richness	7.3	8.1	2.1	4.5 (p<0.000)
P Richness	6.1	5.8	2.7	13.1 (p<0.000)
T Richness	4.7	3.7	4.0	149.0 (p=0.62)

Figure 10 Box plot comparison of richness of EPT component insect orders across Mined, Unmined (from Pond et al. 2008), and WVDEP Reference sites (Mountain Spring bioregion), with mean values and Mann-Whitney U-tests results included (table insert). Where Mined sites contained high E and P richness, conductivity was always less than 500 μS/cm. By using number of genera rather than % of assemblage, both #of E and # of P are significantly different, but there is greater discrimination with # of E.

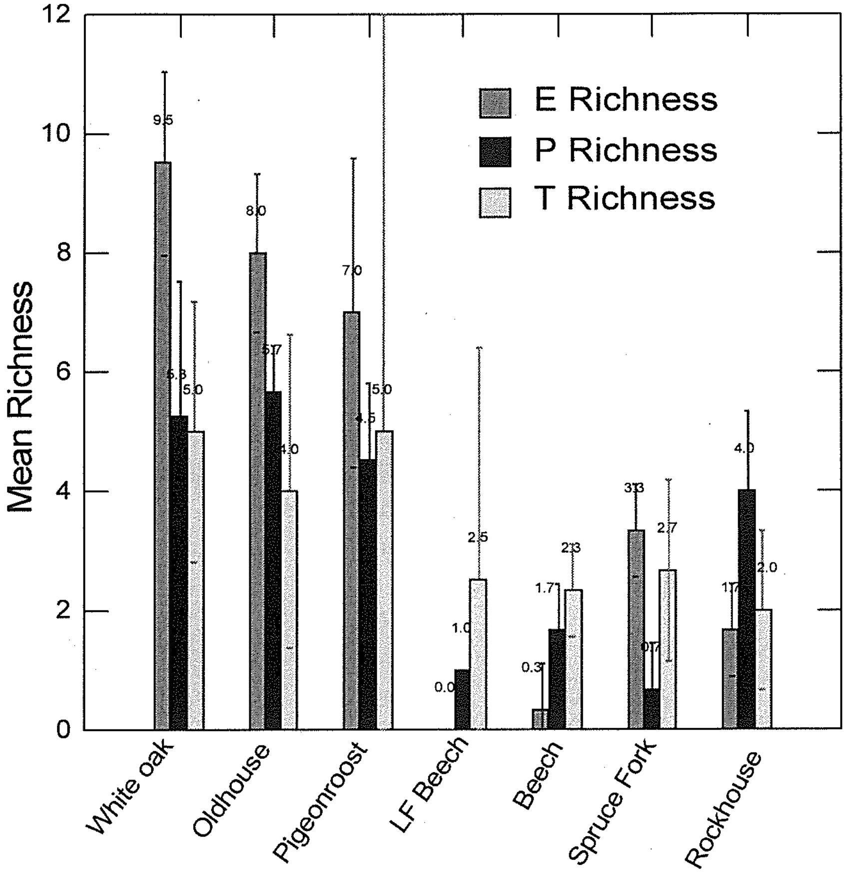


Figure 11. Mean richness (whisker = SD) of E, P, and T genera in streams draining Spruce No. 1 mine area, and adjacent mined streams at Dal-Tex, Rockhouse, and mainstem Spruce Fork near White Oak. All three insect orders exhibit loss of taxa at adjacent mined sites. Data are based on 1 m^2 riffle samples and 200 fixed-count subsamples (EPA R3 data from PEIS (1999-2000, supplemented in 2007)).

A1.5.3 Use of Total Abundance or Density as Response Metrics

Region III does not believe that total macroinvertebrate abundance or density are appropriate metrics to determine effects, as the response of these metrics can be highly variable, and an increase in these metrics is not always "beneficial." While certain pollutants can sharply decrease invertebrate abundance (e.g., sediment), it is well known that this measure is highly variable across both impacted sites and undisturbed sites and has been shown to be unreliable for impact assessment. In fact, it has been known for decades that higher densities often result from a pollution signature. For example, discharges from improperly operated sewage treatment plants or confined animal feeding operations can release organic waste providing an abundance of food for some tolerant, opportunistic macroinvertebrates (e.g., tolerant worms, midgeflies, and hydropsychid caddisflies). This increased abundance can result from discharges having excess nutrients, organic wastes, or other pollutants. Thus, the idea that coal mining is not adverse because it increases "total" abundance below the mine operation (at the expense and demise of sensitive, indigenous taxa) is flawed.

A1.6 EPA's Focus on Sensitive Taxa

EPA's analyses focus on the effects of mining on sensitive taxa because these taxa reflect unimpaired conditions, as found at regional reference sites. To the extent sensitive taxa make up the majority of the native wildlife expected in natural streams across the ecoregion (and in which WVDEP has designated as reference quality streams), then it is appropriate to consider those sensitive taxa. Many of the sensitive taxa that EPA is concerned about are naturally ubiquitous across the region, not rare, or endangered. Therefore, when results show that nearly all of these sensitive animals are extirpated (as in similar MTM streams and Dal-Tex), there is a significant deviation from natural or baseline conditions. While it is true that sensitive taxa can be affected by any land use disturbance or other permitted activity, the preponderance of evidence points to declines and extirpation of both sensitive and common native taxa downstream of MTM operations similar to the Spruce No. 1 proposal.

Shifts in community structure are indicators of adverse effects not only to macroinvertebrate communities, but also to broader ecosystem functions, including organic matter transport, productivity in streams and food web interactions. Data from Hartman et al (2005), and Pond et al. (2008) showed significant "shifts', among other strong evidence, to demonstrate impairment downstream of MTM/VF. Furthermore, WVDEP has acknowledged that a "shift" in the macroinvertebrate community *can* constitute impairment, stating in their published 2008 Integrated List Report, "A "shift" in the benthic macroinvertebrate community of a stream can constitute biological impairment pursuant to 47CSR2 – 3.2.i, and the WVSCI (recognized as a "best science method" in the MTM/VF EIS) provides a sound scientific basis for assessment."

Pond et al. (2008) not only found more than "diminished numbers of certain genera of mayflies," but reported strong evidence of (1) impacts to many other forms of benthic wildlife (including stoneflies and caddisflies), (2) impacts based on 15 out of 17 different community metrics tested, and (3) impacts based on the WVSCI. The published Pond et al. article found many EPT taxa were extirpated or severely reduced downstream of MTM operations (e.g., see Appendix 3 in EPA study). The EPA authors do mention significant "shifts" in composition, but actually point to the WVSCI scores (and for comparison, the more accurate genus-level metrics) as indicating probable "violations of narrative standards". Declining WVSCI scores are in direct response to these "shifts". Hence, "shifts" in community structure can indicate that benthic populations are harmed and eradicated downstream of mining operations.