

1 RUNNING HEAD: Emergence production in valley filled Appalachian streams
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8 **Estimating benthic secondary production from aquatic insect emergence in streams**
9 **affected by mountaintop removal coal mining, West Virginia, USA**
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47 **Abstract:**

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49 Mountaintop removal and valley fill (MTR/VF) coal mining recounours the Appalachian
50 landscape, buries headwater stream channels, and degrades downstream water quality. The goal
51 of this study was to compare benthic community production estimates, based on seasonal insect
52 emergence, between mined and forested streams in the Twentymile Creek watershed, WV
53 (USA). We also assessed the relationship between structural and functional indicators by
54 comparing our production estimates to traditional bioassessment measures. Emergence traps
55 were deployed seasonally for 2-4 weeks beginning in Autumn 2007 along 100-m reaches in each
56 of five mined and five forested streams. The study reaches in the mined streams were located at
57 varying distances downstream of their respective valley fills. Benthic community production
58 was calculated using published length-mass equations and emergence:production ratios. No
59 differences in seasonal emergent density (indiv. $m^{-2} d^{-1}$), biomass ($mg m^{-2} d^{-1}$) or annual
60 secondary production ($g AFDM m^{-2} y^{-1}$) were detected between treatments. Annual secondary
61 production estimates for mined streams were highly variable and averaged $29.6 g AFDM m^{-2} y^{-1}$,
62 but ranged from $1.51 g AFDM m^{-2} y^{-1}$ in the stream nearest to its valley fill to $65.69 g AFDM m^{-2} y^{-1}$
63 y^{-1} in another stream that was 1 km downstream from its fill. Production of forested streams
64 was more consistent with an average of $20.42 g AFDM m^{-2} y^{-1}$ and ranged only from $13.81-$
65 $27.17 g AFDM m^{-2} y^{-1}$. Annual production estimates were not correlated with benthic
66 community index scores, component metrics, or habitat assessment scores. Only EPT
67 production estimates of were significantly correlated with structural endpoints. Conductivity of
68 mined streams was $>30X$ greater than forested streams and contributed to strong differences in
69 emergence composition. Chironomids alone accounted for $>80\%$ of production in mined
70 streams while forested streams had significantly higher EPT production. Measures of stream

71 ecosystem function, including secondary production, can provide a more holistic stream
72 assessments. Prior to their widespread application as indicators of stream health, however,
73 studies are needed to further develop robust response functions across disturbance gradients from
74 multiple stressors.

75 **Keywords:** Appalachian, bioassessment, Chironomidae, conductivity, ecosystem function, EPT,
76 indicator, stream assessment, valley fill

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78 **INTRODUCTION**

79 Coal-fired power plants currently generate about half the electricity used in the United
80 States, despite changing energy policies that promote greater use of alternative fuels. In 2007
81 nearly one quarter of all U.S. coal came specifically from the Central Appalachian Mountains,
82 primarily eastern Kentucky, southern West Virginia, and Virginia (USDOE 2008a,b). Much of
83 the coal in this region is highly valued for its relatively high heating capacity and low sulfur
84 content. Low sulfur coal is in demand as a result of more stringent sulfur dioxide (SO₂)
85 emissions standards established by the Federal Clean Air Act Amendment of 1990 (Fox 1999).

86 Coal mining in the central Appalachians has increasingly shifted from underground or
87 contour mining to the use of large scale mountaintop removal and valley filling (MTR/VF)
88 operations as a result of the increased demand and economic viability. By the MTR/VF method
89 of extraction, explosives and heavy equipment are used to completely remove overlying
90 mountaintops to access coal seams. The excess overburden, or spoil, is then placed in the
91 adjacent valleys, creating a valley fills that permanently bury headwater stream channels.
92 Though such fills often result from other forms of mining or development, by far the largest are
93 associated with MTR/VF activities. From 1985 to 2001 alone, an estimated 724 stream miles
94 were buried under fills in Appalachia (USEPA 2005).

95 Aside from direct loss of stream channels by burial, there are numerous indirect effects of
96 MTR/VF on downstream resources. Sediment control ponds are constructed below valley fills to
97 facilitate removal of particulates, though fine sediments can still be elevated downstream (Wiley
98 et al. 2001). Movement of water through unconsolidated fill material also leeches ions and
99 results in highly elevated total dissolved solids (measured as specific conductance) downstream
100 (e.g., USEPA 2005, Johnson et al. 2010). Stream conductivity at filled sites can be 100X greater

101 than for unmined streams in the region (USEPA 2005) (i.e., from ca. 50 $\mu\text{S cm}^{-1}$ to 5,000 $\mu\text{S cm}^{-1}$) and evidence suggests that conductivity can remain high for decades after fill construction
102 ¹) and evidence suggests that conductivity can remain high for decades after fill construction
103 (Merricks et al., 2007). Other adverse effects include stabilized water temperatures, elevated
104 base flow, increased flood potential, and impaired benthic macroinvertebrate communities
105 (Wiley et al. 2001, USEPA 2005, Hartman et al. 2005, Pond et al. 2008, Griffith et al. 2012).

106 *Litigation and need for functional measures*

107 Mining coal by MTR/VF is highly controversial and the practice has been the subject of
108 much litigation. In a 2007 federal court ruling (Ohio Valley Environmental Coalition v.
109 USACE, Southern District, West Virginia), the need for measurement of stream ecosystem
110 functions was highlighted because appropriate mitigation practices require replacement of
111 functions lost by burial of headwater streams. Yet functions are not typically measured, neither
112 in the streams prior to burial nor in resulting mitigation areas. Stream assessment methods have
113 instead relied solely on conventional structural measures (e.g., EPA rapid bioassessment
114 protocols [Barbour et al. 1999]) that focus primarily on species abundance, habit, feeding mode,
115 and assemblage composition. Qualitative measures of habitat structure are also typically
116 incorporated. These structural measures of stream health have a proven history in stream
117 assessment and are widely applied by state agencies to report on stream condition as required by
118 §305(b) of the Clean Water Act. Structural assessment measures, however, are only snapshots in
119 time that may say little of underlying ecological processes. Measures of both structure and
120 function are needed for more holistic characterization of stream health (e.g., Gessner and
121 Chauvet 2002, Young et al. 2008, Fritz et al. 2010, Clapcott et al. 2011).

122 Stream ecosystem functions are typically expressed as rates and include a wide variety of
123 measures (e.g., metabolism, nutrient uptake, organic matter decomposition, primary and

124 secondary production) that describe energy flow and how materials are stored, processed, or
125 transported within a system. Functional measures have been widely used in basic research to
126 characterize ecosystem processes worldwide and to assess functional responses to various
127 disturbance types and experimental manipulations (e.g., Carpenter et al. 2001, Peterson et al.
128 2001, Cross et al. 2006). Most functional measures are, however, more time, labor, or cost
129 intensive than structural measures so they have not been widely applied by regulatory agencies
130 for stream assessment purposes. Compared to studies using structural measures, fewer studies
131 have measured functions in response to anthropogenic disturbance where multiple stressors may
132 be present, and most have focused on organic matter decomposition (Guessner and Chauvet
133 2002, Dangles et al. 2004, Hagen et al. 2006, Fritz et al. 2010, Clapcott et al. 2011) rather than
134 functions that can be more difficult to measure, such as stream metabolism (but see Bunn et al.
135 1999, Fellows et al. 2006, Young et al. 2008, Feio et al. 2010, Clapcott et al. 2011) or secondary
136 production (but see Kedzierski and Smock 2001, Shieh et al. 2002, Carlisle and Clements 2003,
137 Runck 2007, Woodcock and Huryn 2008).

138 Secondary production measures the rate of biomass accumulation in a give area through
139 time for a given species or community (e.g., Benke and Huryn 2006). Though several different
140 methods may be used to calculate production, all require multiple specimen collections over the
141 entire developmental stage to measure density and biomass of the population(s) of interest
142 (Benke and Huryn 2006). Annual production studies of temperate stream invertebrates, for
143 example, typically involve monthly or bimonthly sampling. Suggested rapid alternatives to
144 actual measurement of secondary production (reviewed by Benke and Huryn 2006) include
145 application of an assumed production to biomass ratio (P:B) to measures of invertebrate biomass
146 or use of multiple regression models that estimate production based on variables such as

147 temperature, discharge, biomass, or maximum body size. A third rapid approach is the use of
148 emergent aquatic insect biomass. Statzner and Resh (1993) demonstrated the significant
149 relationship between emergent biomass and benthic secondary production for several European
150 streams. Studies that have measured both emergence and larval production of aquatic insects
151 have further indicated that emergence to production ratios (E:P) are relatively constant across
152 taxonomic groups so that emergence data alone can provide accurate estimates of larval
153 production (Poepperl 2000).

154 The objectives of this study were to estimate annual benthic production using seasonal
155 emergence data and to compare insect emergence in mined and forested headwater streams.
156 Specifically, we compared seasonal abundance, biomass, production, and composition of
157 emergent insects. We also aimed to evaluate use of emergence as a functional assessment
158 approach by comparing results with traditional structural measures for stream assessment.

159 **Methods**

160 *Study Area*

161 The Twentymile Creek watershed (ca. 225 km²) is located in southern West Virginia,
162 USA and drains into the Gauley River. The watershed has a trellised drainage and lies within the
163 Dissected Plateau of the Central Appalachians [Level IV Ecoregion (69d); Woods et al. 2002].
164 The ecoregion is unglaciated, consisting of steep ridges and narrow valleys and is underlain with
165 Pennsylvanian sandstone, shale, siltstone, and coal. Soils are comprised of moderately fertile
166 Ultisols and Inceptisols (Woods et al. 1999). The watershed was clearcut in the early 1900s and
167 some historic contour and underground coal mining has taken place in the upper portion of the
168 watershed (Green et al. 2000). The majority of MTM/VF coal has taken place in the upper
169 portion of the watershed in the last 20 years. Vegetation of forested subcatchments now consists

170 primarily of mixed hardwoods, including oaks (*Quercus* spp.), maples (*Acer* spp.), tulip poplar
171 (*Liriodendron tulipifera*) and beech (*Fagus grandifolia*), whereas mined subcatchments are
172 primarily barren or grasslands, with patchy forest in the lower portions. The upper Twentymile
173 watershed is uninhabited while the lower portion is low density residential. Numerous gas wells
174 also exist throughout the watershed.

175 Ten subcatchments were chosen within the larger Twentymile watershed. We used a
176 paired watershed design where five subcatchments drained nearly 100% forested land and five
177 subcatchments drained MTR/VF operations (Table 1). Stream reaches (100 m) with perennial
178 surface flow were chosen for study near the base of each subcatchment. Our forested reaches
179 show legacy effects of logging and have gas wells and access roads within the catchments, but
180 these streams are representative of the least disturbed condition within the study region. Our
181 mined stream reaches represent a gradient of mining disturbance; two were upstream of sediment
182 ponds (which were positioned at the base of the subcatchments) and three were at varying
183 distances downstream of ponds (Table 1). Watershed sizes ranged from 0.59-5.27 km²
184 (treatment medians: forest = 1.62 km²; mined = 1.43 km²).

185 *Emergence and Production*

186 Aquatic insect emergence was measured seasonally in each stream by deploying
187 emergence traps for 2-4 weeks beginning Autumn 2007 (21 Sept. – 8 Oct.). Other deployment
188 dates were 24 Jan. – 12 Feb. (Winter), 1 April – 16 April (Spring), and 14 June – 16 July
189 (Summer), 2008. Seasonal collection dates were staggered among streams because not all could
190 be sampled in one day, but differences in start and end dates among streams were all ≤ 4 d each
191 season.

192 During each of the four collection periods, three emergence traps were placed in each of
193 the 10 stream reaches, with two traps in riffle habitat and one in pool (n = 120 total
194 traps/samples). Mean percentage riffle habitat within study reaches was 67.9% (range 44.5-
195 80%), thus habitats were sampled roughly in proportion to their occurrence. Emergence traps
196 were enlarged versions of Banks et al. (2007) and consisted of hinged wooden frames that, when
197 opened, covered a rectangular area of 0.28 m² of streambed (Banks et al. [2007] covered 0.19
198 m²). Plastic capture wells were placed between frame crosspieces and filled with preservative
199 consisting of 2/3 glycerol and 1/3 10% buffered formalin with soapy water added to reduce
200 surface tension. Frames were then completely enclosed within 250- μ m mesh. Canvas skirts
201 sewn to the bottom of the netting were anchored to the stream bottom with cobbles. A sheet of
202 clear plastic was also clipped over the upper 1/3 of the trap to prevent rainwater from overflowing
203 and diluting capture wells. Further details for trap construction are provided by Banks (2005).
204 Upon collection, capture wells were sieved (250- μ m) and preserved in 70% ethanol. Contents of
205 each trap were preserved separately. In the laboratory, emergent aquatic insects were identified
206 to Order or Suborder and measured to the nearest mm under a dissecting microscope (10X).
207 Length measures were then converted to ash-free dry mass (AFDM) using published length-dry
208 weight regression equations for adult aquatic insects (Sabo et al. 2002) with an ash conversion
209 factor of 15% (Smock 1980).

210 Daily emergent density (indiv. m⁻² d⁻¹) and biomass (mg m⁻² d⁻¹) were calculated for each
211 trap by dividing totals by the number of days in the incubation period. Seasonal emergent
212 production was then calculated by multiplying daily emergence biomass by the total number of
213 days in each season and annual emergence production was calculated as the sum of seasonal
214 emergence production values. Annual emergent production estimates were then used to

215 calculate annual benthic production by application of a mean emergence biomass to larval
216 production ratio (E:P) of 18.3% (Poepperl 2000), a mean value similar to those reported in other
217 studies that have compared stream insect emergence with benthic production (Hall et al. 1970,
218 Speir and Anderson 1974, Jackson and Fisher 1986, Statzner and Resh 1993). Emergent density,
219 biomass, and all production estimates were calculated for both all taxa combined and individual
220 taxonomic groups.

221 *Structural Assessment Measures*

222 Benthic macroinvertebrates were collected from each stream in Spring (April) and
223 Summer (July) 2008 using a kicknet (0.5-m-wide, 595 μm mesh). Four 0.25 m² kick samples
224 were collected from riffle habitat of each stream and composited (1 m² total). In the laboratory,
225 preserved samples were randomly subsampled to obtain 200 \pm 20% individuals and all
226 organisms were identified to genus level. Resulting data were used to calculate the Genus-Level
227 Index of Most Probable Stream Status (GLIMPSS; Pond et al. 2008). This multimetric index is
228 currently used by the West Virginia Department of Environmental Protection and the US
229 Environmental Protection Agency (EPA) Region 3 for assessing stream health in West Virginia.
230 Index metrics include: total generic richness; intolerant richness; Ephemeroptera generic
231 richness; Plecoptera generic richness; clinger generic richness; Genus biotic index; %
232 Ephemeroptera; % Orthocladinae; and % 5 dominant genera.

233 We measured pH, conductivity ($\mu\text{S}/\text{cm}$), dissolved oxygen (mg/l), and in each stream
234 during each season using a portable multiprobe (Hydrolab Quanta; Hydrolab Corp., Austin, TX).
235 Water temperature was measured at 4-h intervals with StowAway TidbiTH temperature loggers

236 (OnsetH Computer Corp., Bourne, MA). Stream discharge was measured each season using the
237 velocity area method and we used an electronic flow meter (Marsh-McBirney Flo-mate,
238 Loveland, CO) to measure velocity.

239 Habitat was qualitatively scored at each site using both the US EPA Rapid Bioassessment
240 Protocol (RBP) for high gradient streams (Barbour et al. 1999) and the Riparian, Channel, and
241 Environmental (RCE) Inventory (Peterson 1992). The RBP has been used by regulatory
242 agencies to assess mitigation for MTM/VF and scores each of the following variables 0-20 each
243 for total possible score of 200: epifaunal substrate, embeddedness, velocity/depth regime,
244 sediment deposition, channel flow status, channel alteration, frequency of riffles, bank stability,
245 vegetative protection, and riparian vegetative zone width. The RCE scores 16 variables, each in
246 four scoring categories that are differentially weighted, for a total possible score of 360. Total
247 scores are then used to classify streams from 1 (excellent) to 5 (poor) with recommended
248 management actions for each class. RCE variables are: landuse beyond riparian zone, riparian
249 zone width, completeness of riparian zone, riparian vegetation within 10 m of channel, retention
250 devices, channel structure, channel sediments, stream-bank structure, bank undercutting, stony
251 substrate feel and appearance, stream bottom, riffles and pools, aquatic vegetation, fish, detritus,
252 and macrobenthos.

253 *Data Analyses*

254 Preliminary results indicated no significant differences between pool and riffle traps for
255 emergent insect abundance (paired t-test, D.F. = 28, $p = 0.62$, [two riffle traps averaged when
256 both were recovered]) or biomass ($p = 0.52$) within streams where traps from both habitats were
257 recovered. Pool and riffle samples were therefore averaged for each stream and collection date
258 for calculations of emergent density, biomass, and production. Emergent insect abundance and

259 biomass values were $\log(x+1)$ transformed and tested for differences using a generalized linear
260 model (PROC GLM, SAS 9.1; SAS Institute, Cary, North Carolina) with season and treatment as
261 factors and stream nested within treatment. Streams were nested within each treatment because
262 each stream belonged to only one treatment group (e.g., mined or forested). Significant
263 treatment effects were followed with Tukey multiple comparisons for that factor. Differences in
264 assemblage composition between treatments were first tested using the multi-response
265 permutational procedure (MRPP, PC-ORD v. 4.25, McCune and Mefford 1999) which has
266 proven useful in comparing species composition data (Biondini et al. 1985; Zimmerman et al.
267 1985). Sensitive taxa within the Orders Ephemeroptera, Plecoptera, and Trichoptera were
268 combined into a metric (EPT) and the combined density and biomass were tested using a
269 generalized linear model as described above. Calculated production estimates and functional
270 metrics (e.g. EPT production) were correlated with structural assessment measures by Pearson
271 correlations (r_P) or, if data failed to meet the assumption of normality after data transformation,
272 Spearman correlation (r_S) (SigmaStat v. 3.0, SPSS, Inc.).

273 **Results**

274 Stream conductivity values ranged from 49-2513 $\mu\text{S}/\text{cm}$ over the study period and the
275 mean of mined streams (1630 $\mu\text{S}/\text{cm}$) was >30X higher than that of forested streams (52
276 $\mu\text{S}/\text{cm}$)(Table 2). pH of forested streams were slightly acidic to neutral (range: 5.87-6.94),
277 whereas mined streams were more alkaline (range: 7.04-8.89). Mined streams were also, on
278 average, ca. 1°C warmer than forested streams over the study period. Stream discharge and
279 dissolved oxygen were similar between treatment groups (Table 2). The average
280 macroinvertebrate index score (GLIMPSS) for forested streams was more than double that of

281 mined streams and habitat assessment scores (RBP and RCE) were also consistently higher in
282 forested streams, indicating impairment of mined streams (Table 2).

283 Of the 120 traps deployed during the study, 92 were successfully recovered (77%) (Table
284 3). Traps were sometimes knocked down, either by animals or high stream flows. Trap losses
285 were highest in summer (47%) and lowest in winter (7%), and trap losses were similar between
286 mined and forested streams. There were no traps recovered for one forested stream in Autumn
287 (Peters) and three streams in Summer (one forested [Ash], two mined [Buckles and Lost]).

288 There were significant differences in insect emergence among seasons for both density
289 and biomass (Table 4), with greatest emergence in summer and lowest in winter (Figure 1).
290 There was, however, no significant treatment effect for either density or biomass of emergent
291 insects over the study period. There were differences among streams within treatments
292 (stream[treatment]) that resulted largely from differences between mined streams with study
293 reaches located upstream of sediment ponds versus those with study reaches located downstream
294 of sediment ponds (Figure 1, Table 4).

295 Emergence was often highly variable among streams. In winter, emergent density and
296 biomass ranged from only 0.90 indiv. $m^{-2} d^{-1}$ and 0.26 mg $m^{-2} d^{-1}$, respectively, in Lost Creek
297 (mined, upstream of sediment pond and closest to a valley fill) to 17.41 indiv. $m^{-2} d^{-1}$ and 9.34
298 mg $m^{-2} d^{-1}$ in Buckles Branch (mined, downstream of sediment pond). Winter densities averaged
299 only 2.81 and 5.21 indiv. $m^{-2} d^{-1}$ for forested and mined streams, respectively (Fig. 1). Winter
300 biomass averaged 1.26 mg $m^{-2} d^{-1}$ in forested streams and 2.81 mg $m^{-2} d^{-1}$ in mined streams.
301 During peak summer emergence, no traps were recovered in either Lost Creek or Buckles
302 Branch, but emergence rates were lowest in Beech Fork (11.53 indiv. $m^{-2} d^{-1}$, 1.98 mg $m^{-2} d^{-1}$),
303 the other mined site upstream of a sediment pond, and highest in Sugarcamp Creek (171.21

304 indiv. $\text{m}^{-2} \text{d}^{-1}$, 91.10 $\text{mg m}^{-2} \text{d}^{-1}$), which like Buckles, is a mined site located >1 km downstream
305 of the valley fill and sediment pond. Summer density and biomass averaged 91.95 indiv. $\text{m}^{-2} \text{d}^{-1}$
306 and 22.93 $\text{mg m}^{-2} \text{d}^{-1}$, respectively in forested streams, whereas mined streams averaged 75.77
307 indiv. $\text{m}^{-2} \text{d}^{-1}$ and 34.44 $\text{mg m}^{-2} \text{d}^{-1}$ (Fig. 1). Emergence rates from forested and mined streams in
308 both autumn and spring were intermediate to those in winter and summer. The lowest
309 emergence rates in autumn and spring were from Lost Creek, where emergence rates (Autumn:
310 density = 0.97 indiv. $\text{m}^{-2} \text{d}^{-1}$, biomass = 0.34 $\text{mg m}^{-2} \text{d}^{-1}$; Spring: density = 1.40 indiv. $\text{m}^{-2} \text{d}^{-1}$,
311 biomass = 0.44 $\text{mg m}^{-2} \text{d}^{-1}$) were >20X lower than the season average of all streams combined.

312 For annual benthic production calculations, when no traps were recovered in a stream (4
313 occasions) we used the treatment average for that season. However, for one mined stream in
314 Summer (Lost) we used the value of the other mined site upstream of a sediment pond (Beech)
315 because of differences in emergence at sites upstream and downstream of sediment ponds.

316 Conversion of seasonal emergent biomass to benthic production resulted in annual averages of
317 20.42 g AFDM $\text{m}^{-2} \text{yr}^{-1}$ for forested streams and 29.60 g AFDM $\text{m}^{-2} \text{yr}^{-1}$ for mined streams.

318 Secondary production from mined streams showed much greater variability, however, ranging
319 from 1.51 g AFDM $\text{m}^{-2} \text{yr}^{-1}$ (Lost Creek) to 65.69 g AFDM $\text{m}^{-2} \text{yr}^{-1}$ (Buckles Branch), whereas
320 forested streams ranged just 13.81 g AFDM $\text{m}^{-2} \text{yr}^{-1}$ (Peters Creek) to 27.17 g $\text{m}^{-2} \text{yr}^{-1}$ (Jacks
321 Branch; Fig. 2).

322 While there was no treatment effect on emergent insect abundance, biomass, or
323 production, there were significant differences in taxonomic composition. Chironomids
324 dominated emergence in all seasons and generally comprised at least half of all insect production
325 in each stream (Fig. 2, Appendix). Forested streams had greater production attributed to EPT
326 taxa, while production in mined streams was dominated by Chironomidae. Differences in

327 composition between forested and mined streams were significant during both Spring (MRPP, A
328 = 0.09, $p = 0.021$) and Summer ($A = 0.12$, $p = 0.037$) emergence periods. There were no
329 significant composition differences in either Autumn ($A = 0.03$, $p = 0.185$) or Winter ($A = 0.008$,
330 $p = 0.39$) when emergent samples consisted largely of Chironomidae and winter stoneflies.
331 There was also a highly significant treatment effect when only emergent density and biomass of
332 EPT taxa were considered (Table 5). In Spring, EPT density and biomass of forested streams
333 averaged 1.97 indiv. $m^{-2} d^{-1}$ and 5.06 mg $m^{-2} d^{-1}$, respectively, whereas mined streams averaged
334 only 0.16 indiv. $m^{-2} d^{-1}$ and 0.88 mg $m^{-2} d^{-1}$. Differences were even greater in Summer when
335 forested streams averaged 9.62 indiv. $m^{-2} d^{-1}$ and 11.35 mg $m^{-2} d^{-1}$, and mined streams averaged
336 0.96 indiv. $m^{-2} d^{-1}$ and 1.00 mg $m^{-2} d^{-1}$. Though two mined streams, Sugarcamp and Buckles, had
337 the highest annual production measured in this study (52.27 g AFDM $m^{-2} yr^{-1}$ and 65.69 g
338 AFDM $m^{-2} yr^{-1}$, respectively), Nematocera (primarily Chironomidae) alone accounted for ca.
339 87% of benthic production (45 and 57.8 g AFDM $m^{-2} yr^{-1}$, respectively) in each of these streams
340 (Fig. 2). In the forested streams chironomid production never accounted for more than 54% (in
341 Neil) of total insect production, while EPT taxa accounted for, on average, 48% of production
342 (Fig. 2, Appendix). EPT production in mined streams remained consistently low across seasons,
343 the average ranging from only 0.30 g AFDM $m^{-2} yr^{-1}$ (Winter) to 0.48 g AFDM $m^{-2} yr^{-1}$
344 (Summer), and was comprised primarily of nemourid stoneflies and hydropsychid caddisflies.
345 Mayflies (Ephemeroptera) were conspicuously rare in mined streams where average production
346 was only 0.13 g AFDM $m^{-2} yr^{-1}$ (0.4% of total), compared to 4.4 g AFDM $m^{-2} yr^{-1}$ (22% of total)
347 in forested streams.

348 Annual secondary production estimates for each stream were not correlated with any of
349 the 9 physiochemical and structural assessment measures included in this study (from Table 2,

350 Pearson correlation coefficients range: -0.29 to 0.48; $p > 0.05$). Annual production of combined
351 EPT taxa, however, was significantly correlated with 7 of the 9 variables: conductivity ($rS = -$
352 $.939, p = <0.001$), pH ($rP = -0.785, p = 0.007$), temperature ($rP = -0.744, p = 0.014$), degree days
353 ($rP = -0.798, p = 0.006$), GLIMPSS ($rP = 0.815, p = 0.004$), RBP ($rP = 0.768, p = 0.010$), and
354 RCE ($rP = 0.769, p = 0.009$).

355 **DISCUSSION**

356 Our findings provide an example of how structural and functional measures can provide
357 unique insights to stream ecosystem health. We found strong structural differences (e.g., species
358 composition) between mined and forested streams, but secondary production estimates were
359 highly variable and were not, on average, different between treatments. Variability of mined
360 streams appeared to be related to distance from valley fills, with production generally increasing
361 with distance from valley fills. Total benthic community production estimates correlated poorly
362 with structural assessment parameters. Only production of sensitive EPT taxa correlated well
363 with the commonly used structural measures. The relative absence of sensitive taxa, particularly
364 Ephemeroptera, and high TDS we observed in mined streams supports previous studies in
365 Appalachian streams affected by coal mining activities (Hartman et al. 2005, Merricks et al.
366 2007, Pond et al. 2008, Pond 2010, Griffith et al. 2012). Further mechanistic studies (*sensu*
367 Buchwalter and Luoma 2005, Xie et al. 2010) of ecophysiology are needed to fully explain
368 sensitive species losses in these mined streams.

369 Lack of overall difference in emergence abundance, biomass, and production between
370 treatments results from prevalence of pollution tolerant chironomids in mined streams that was
371 offset with a corresponding reduction of sensitive EPT taxa. When insect emergence rates were
372 averaged by treatment, we found no differences within any season. We found expected seasonal

373 peak emergence in summer and low in winter, however the similarity of emergence rates in
374 autumn and spring was surprising. Autumn emergence, however, consisted almost entirely of
375 chironomids, and had more, smaller individuals, whereas Spring samples consisted of fewer,
376 larger individuals, and more EPT taxa (Appendix). Summer samples, particularly among mined
377 streams, had high variability resulting largely from trap losses during that period.

378 Our mean benthic community production estimate of $25 \text{ g m}^{-2} \text{ yr}^{-1}$ across all streams
379 would be considered fairly typical of lotic systems (e.g., Huryn and Wallace 2000), though
380 production in mined streams was highly variable and appeared to be related to distance from the
381 valley fill. The two mine sites upstream of the sediment ponds (Lost and Beech) had the lowest
382 secondary production measured here, whereas the two mined sites furthest downstream of fills
383 (Buckles and Sugarcamp) had significant higher production than reference streams. Our
384 calculated annual production of $1.5 \text{ g AFDM m}^{-2} \text{ yr}^{-1}$ for mined Lost Creek is among the lowest
385 reported for streams (Huryn and Wallace 2000). The sample reach in Lost was upstream of the
386 sediment pond and had the lowest habitat score (e.g., RBP = 119) of any stream in this study.
387 This study site was also closest to the valley fill, had highly unstable channel with substrates
388 consisting of gravel, sand, and fines that had washed down from the fill. Riparian vegetation
389 was also largely lacking and the stream reach would be considered highly impaired. Beech
390 Creek, the other mined stream upstream of the sediment pond had more a more typical
391 production value of $10.8 \text{ g AFDM m}^{-2} \text{ yr}^{-1}$, though still second lowest of the streams measured
392 and comprised primarily of Chironomidae. Unlike Lost Creek, Beech Creek has an intact
393 riparian zone and larger, more stable substrates that improved habitat quality relative to Lost. In
394 many cases, a single sediment pond will serve multiple valley fills and the length of stream
395 between the toe of fills and sediment ponds can extend hundreds of meters.

396 Contrasting the low production of Lost and Beech Creeks, our estimated benthic
397 production in two mined streams located furthest from valley fills had much higher production
398 than any other stream, despite no obvious increase in basal resources, such as periphyton or
399 particulate organic matter (unpublished data). Higher secondary production in Sugarcamp and
400 Buckles, was largely attributed to chironomid production values of 45 and 58 g AFDM m⁻² yr⁻¹,
401 respectively, which comprised 87% of total benthic production in each stream. These estimated
402 values are similar to the high chironomid production values reported for Sycamore Creek, AZ
403 (Jackson and Fisher 1986) and snag habitats of the Ogeechee River, GA (Benke 1998), which
404 may represent extreme examples of high midge production for undisturbed streams.
405 Furthermore, our estimates are very similar to those of Runck (2007) who calculated chironomid
406 production of 59.5 g AFDM m⁻² in an industrially contaminated, nutrient-enriched stream.
407 Similarly, Shieh et al. (2002) found higher production of non-tanypodine chironomids (59.2 g
408 AFDM m⁻² y⁻¹) in a stream receiving treated wastewater and attributed excess production to
409 increased nutrients and reduced predation pressure. In these mined streams, high chironomid
410 production could also have resulted from their tolerance to degraded water quality or from
411 indirect effects such reduced competition and predation pressure. Temperature significantly
412 influences macroinvertebrate growth and production (e.g., Huryin and Wallace 2000) and
413 Sugarcamp creek averaged ca. 1.5 ° warmer than other streams and accumulated 430 more DD.
414 However, the stream with the highest production, Buckles Creek, had accumulated lowest degree
415 days among the mined streams (Table 2). Predators may be less abundant as a result of degraded
416 water quality (e.g., high TDS) or lack their preferred prey. When prey resources shift from
417 larger bodied taxa (e.g., EPTs) to smaller-bodied midges, predators would theoretically have to
418 expend more energy searching for a greater number of smaller individuals. More food web

419 studies are needed, however, that document food web energetics of disturbed streams (i.e.,
420 Runck 2007).

421 The relationship between ecosystem structure and functions has received much attention
422 (e.g., Loreau et al. 2001, Fritz et al. 2010, Isbell et al. 2011). Odum (1985) suggested that
423 ecosystem functions may be less responsive to stress than structural measures such as species
424 diversity, and this has been supported by several researchers (e.g. Schindler 1985, Frost et al.
425 1995, Niyogi et al. 2002). This functional complementarity, or redundancy, can occur when
426 disturbance causes shifts in species composition, yet ecosystem functions remain largely
427 unaffected (e.g., Frost et al. 1995), suggesting an inherent stability of process. We found that
428 traditional stream assessment measures, such as EPT metrics and habitat scores, were
429 consistently responsive to mining disturbance in our study streams, only when considering
430 production of combined EPT taxa did we find correlations with structural indicators. Measures
431 of community production were, however, highly variable among mined sites, whereas values
432 from forested streams were relatively consistent. Though we found no differences in *average*
433 production between treatments, our findings highlight the unique benefits of functional
434 assessment methods. Variability of production estimates at mined sites are likely related to not
435 only water quality and habitat, as structural indicators typically reflect, but also the underlying
436 resource base, food web structure, and system energetics.

437 Studies of anthropogenic disturbance effects on stream ecosystem functions are
438 increasing, but their sensitivity to disturbance may depend on the specific function chosen,
439 stressor(s) present, and system in question. This study was limited to secondary production in a
440 small number of mined streams and further research is needed to fully evaluate utility of
441 functional assessment methods. Measures of organic matter decomposition are among the most

442 straightforward functions to measure and thus far have shown most promise as an indicator of
443 stream health (reviewed in Gessner and Chauvet 2002). Additional studies are needed that
444 further assess strength and direction of functional responses, sensitivity to various disturbance
445 types, and the accuracy and precision measurements across larger spatial scales. Use of
446 functional measures is not intended as a replacement for existing methods used to assess stream
447 health. Rather, they are intended as a complement to provide process information that will aid
448 regulatory and stream mitigation programs to prevent loss of ecosystem functions. Further, no
449 single functional assessment method will properly evaluate ecosystem processes. A suite of
450 functional measures are recommended to provide a more thorough, holistic assessment of stream
451 ecosystem health (Young et al. 2008, Feio et al. 2010, Clapcott et al. 2011).

452

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465 **Literature Cited**

466 Banks, J.L., J. Li, and A.T. Herlihy. 2007. Influence of clearcut logging, flow duration, and
467 season on emergent aquatic insects in headwater streams of the Central Oregon Coast
468 Range. *Journal of the North American Benthological Society* 26:620-632.

469 Banks, J.L. 2005. Influences of clearcut logging on macroinvertebrates in perennial and
470 intermittent headwaters of the Central Oregon Coast Range. MS Thesis, Oregon State
471 University, Corvallis, OR.

472 Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment
473 protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates
474 and fish. Second edition. EPA 841-B-99-002. United States Environmental Protection
475 Agency; Office of Water; Washington, D.C.

476 Benke, A.C., and A.D. Huryn. 2006. Secondary production of macroinvertebrates. In: *Methods*
477 *in Stream Ecology*, 2nd ed., F.R. Hauer and G.A. Lamberti, eds. Academic Press. New
478 York, USA.

479 Benke, A.C. 1998. Production dynamics of riverine chironomids: Extremely high biomass
480 turnover rates of primary consumers. *Ecology* 79:899–910

481 Biondini, M.E., C.D. Bonham, and E.F. Redente. 1985. Secondary successional patterns in a
482 sagebrush (*Artemisia tridentata*) community as they relate to soil disturbance and soil
483 biological activity. *Vegetatio* 60: 25–36.

484 Buchwalter, D.B., and S.N. Luoma. 2005. Differences in dissolved cadmium and zinc uptake
485 among stream insects: Mechanistic explanations. *Environmental Science and*
486 *Technology* 39:498-504.

487 Bunn, S.E., P.M. Davies, and T.D. Mosisch. 1999. Ecosystem measures of river health and their

488 response to riparian and catchment degradation. *Freshwater Biology* 41:333-345.

489 Carlisle, D.M. and W.H. Clements. 2003. Growth and secondary production of aquatic insects
490 along a gradient of Zn contamination in Rocky Mountain streams. *Journal of the North*
491 *American Benthological Society* 22:582-597.

492 Carpenter, S.R., J.J. Cole, J.R. Hodgson, J.F. Kitchell, M.L. Pace, D. Bade, K.L. Cottingham,
493 T.E. Essington, J.N. Houser, D.E. Schindler. 2001. Trophic cascades, nutrients, and lake
494 productivity: Whole-lake experiments. *Ecological Monographs* 71:163-186.

495 Clapcott, J.E., K.J. Collier, R.G. Death, E.O. Goodwin, J.S. Harding, D. Kelly, J.R. Leathwick,
496 and R.G. Young. 2011. Quantifying relationships between land-use gradients and
497 structural and functional indicators of stream ecological integrity. *Freshwater Biology*
498 57:74-90.

499 Cross, W.F., J.B. Wallace, A.D. Rosemond, and S.L. Eggert. 2006. Whole-system nutrient
500 enrichment increases secondary production in a detritus-based ecosystem. *Ecology*
501 87:1556-1565.

502 Dangles, O., M.O. Gessner, F. Guerold, and E. Chauvet. 2004. Impacts of stream acidification
503 on litter breakdown: implications for assessing ecosystem functioning. *Journal of*
504 *Applied Ecology* 41: 365-378.

505 Feio, M.J., T. Alves, M. Boavida, A. Medeiros, and M.A.S. Graca. Functional indicators of
506 stream health: a river-basin approach. *Freshwater Biology* 55:1050-1065.

507 Fellows, C.S., J.E. Clapcott, J.W. Udy, S.E. Bunn, B.D. Harch, M.J. Smith, and P.M. Davies.
508 2006. Benthic metabolism as an indicator of stream ecosystem health. *Hydrobiologia*
509 572:71-87.

510 Fox, J. 1999. Mountaintop removal in West Virginia: an environmental sacrifice zone.

511 Organization and Environment 12:163-183.

512 Fritz, K.M., S. Fulton, B.R. Johnson, C.D. Barton, J.D. Jack, D.A. Word, and R.A. Burke. 2010.

513 Structural and functional characteristics of natural and constructed channels draining a

514 reclaimed mountaintop removal and valley fill coal mine. *Journal of the North American*

515 *Benthological Society* 29:673-689.

516 Fritz, K.M., Johnson, B.R., and D.M. Walters. 2006. *Field Operations Manual for Assessing the*

517 *Hydrologic Permanence and Ecological Condition of Headwater Streams*. EPA/600/R-

518 06/126. U.S. Environmental Protection Agency, Office of Research and Development,

519 Washington, D.C.

520 Frost, T.M., S.R. Carpenter, A.R. Ives, and T.K. Kratz. 1995. Species compensation and

521 complementarity in ecosystem function *In: Linking Species and Ecosystems*. G.C. Jones

522 and J.H. Lawton (eds.). Chapman and Hall, New York, NY.

523 Gessner, M.O., and E. Chauvet. 2002. A case for using litter breakdown to assess functional

524 stream integrity. *Ecological Applications* 12:498-510.

525 Green, J., M. Passmore, and H. Childers. 2000. A survey of the condition of streams in the

526 primary region of mountaintop mining/valley fill coal mining. Appendix D *in*

527 *Mountaintop Mining/Valley Fills in Appalachia Final Programmatic Environmental*

528 *Impact Statement*. EPA Region 3, Philadelphia, PA. EPA 9-03-R-05002.

529 (<http://www.epa.gov/region3/mtntop/eis2005.htm>).

530 Griffith, M.B., S.B. Norton, L.C. Alexander, A.I. Pollard, S.D. LeDuc. 2012. The effects of

531 mountaintop mines and valley fills on the physiochemical quality of stream ecosystems in

532 the central Appalachians: A review. *Science of the total environment* 417-418:1-12.

533 Hagen, E.M., J.R. Webster, E.F. Benfield. 2006. Are leaf breakdown rates a useful measure of

534 stream integrity along an agricultural landuse gradient? *Journal of the North American*
535 *Benthological Society* 25:330-343.

536 Hall, D.J., W.E. Cooper, and E.E. Werner. 1970. An experimental approach to the production
537 dynamics and structure of freshwater animal communities. *Limnology and*
538 *Oceanography* 15:838-928.

539 Hartman, K.J., M.D. Kaller, J.W. Howell, and J.A. Sweka. 2005. How much do valley fills
540 influence headwater streams. *Hydrobiologia* 532:91-102.

541 Huryn, A.D., and J.B. Wallace. 2000. Life history and production of stream insects. *Annual*
542 *Review of Entomology* 45:83-110.

543 Isbell, F., V. Calcagno, A. Hector, J. Connolly, W.S. Harpole, P.B. Reich, M. Scherer-Lorenzen,
544 B. Schmid, D. Tilman, J. van Ruijvan, A. Weigelt, B.J. Wilsey, E.S. Zavaleta, M. Loreau.
545 2011. High plant diversity is needed to maintain ecosystem services. *Nature* 477:199-
546 202.

547 Jackson, J.K., and S.G. Fisher. 1986. Secondary production, emergence, and export of aquatic
548 insects of a Sonoran desert stream. *Ecology* 67:629-638.

549 Johnson, B.R., A. Haas, and K.M. Fritz. 2010. Use of spatially explicit physiochemical data to
550 measure downstream impacts of headwater stream disturbance. *Water Resouces*
551 *Research* 46:W09526

552 Kedzierski, W.M. and L.A. Smock. 2001. Effects of logging on macroinvertebrate production
553 in a sand-bottomed, low-gradient stream. *Freshwater Biology* 46:821-833.

554 Loreau, M., S. Naeem, P. Inchausti, J. Bengtsson, J.P. Grime, A. Hector, D.U. Hooper, M.A.
555 Huston, D. Raffaelli, B. Schmid, D. Tilman, and D.A. Wardle. 2001. Biodiversity and
556 ecosystem functioning: Current knowledge and future challenges. *Science* 294:804-808.

557 McCune, B. and M.J. Mefford. 1999. PC-ORD. Multivariate Analysis of Ecological Data,
558 Version 4. MjM Software Design, Gleneden Beach, OR, USA.

559 Merricks, T.C., D.S. Cherry, C.E. Zipper, R.J. Currie, and T.W. Valenti. 2007. Coal-mine
560 hollow fill and settling pond influences on headwater streams in southern West Virginia,
561 USA. 129:359-378.

562 Niyogi, D.K., W.M. Lewis Jr., and D.M. McKnight. 2002. Effects of stress from mine drainage
563 on diversity, biomass, and function of primary producers in mountain streams.
564 Ecosystems 5:554-567.

565 Odum, E.P. 1985. Trends expected in stressed ecosystems. Bioscience 35: 419-422.

566 Peterson, B.J., W.M. Wollheim, P.J. Mulholland, J.R. Webster, J.L. Meyer, J.L. Tank, E. Marti,
567 W.B. Bowden, H.M. Valett, A.E. Hershey, W.H. McDowell, W.K. Dodds, S.K.
568 Hamilton, S. Gregory, and D.D. Morrall. 2001. Control of nitrogen export from
569 watersheds by headwater streams. Science 292:86-90.

570 Peterson, R.C., Jr. 1992. The RCE: a Riparian, Channel, and Environmental Inventory for small
571 streams in the agricultural landscape. Freshwater Biology 27:295-306.

572 Poepperl, R. 2000. Benthic secondary production and biomass of insects emerging from a
573 northern German temperate stream. Freshwater Biology 44:199-211.

574 Pond, G.J. 2010. Patterns of Ephemeroptera taxa loss in Appalachian headwater streams
575 (Kentucky, USA). Hydrobiologia 641:185-201.

576 Pond, G.J., M.E. Passmore, F.A. Borsuk, L. Reynolds, and C.J. Rose. 2008. Downstream
577 effects of mountaintop coal mining: comparing biological conditions using family- and
578 genus-level macroinvertebrate bioassessment tools. Journal of the North American
579 Benthological Society 27:717-737.

580 Riipinen, M.P., J. Davy-Bowker, and M. Dobson. 2008. Comparison of structural and
581 functional stream assessment methods to detect changes in riparian vegetation and water
582 pH. *Freshwater Biology* doi:10.1111/j.1365-2427.2008.01964.x

583 Runck, C. 2007. Macroinvertebrate production and food web energetics in an industrially
584 contaminated stream. *Ecological Applications* 17:740-753.

585 Sabo, J.L., J.L. Bastow, and M.E. Power. 2002. Length-mass relationships for adult aquatic and
586 terrestrial invertebrates in a California watershed. *Journal of the North American
587 Benthological Society* 21:336-343.

588 Schindler, D.W., K.H. Mills, D.F. Malley, D.L. Findlay, J.A. Shearer, I.J. Davies, M.A. Turner,
589 G.A. Linsey, D.R. Cruikshank. 1985. Long-term ecosystem stress: The effects of years
590 of experimental acidification on a small lake. *Science* 228:1395-1401.

591 Shieh, S.-H., J.V. Ward, and B.C. Kondratieff. 2002. Energy flow through macroinvertebrates
592 in a polluted plains streams. *Journal of the North American Benthological Society* 21:
593 660-675.

594 Smock, L.A. 1980. Relationships between body size and biomass of aquatic insects. *Freshwater
595 Biology* 10:375-383.

596 Speir, J.A., and N.H. Anderson. 1974. Use of emergence data for estimating annual production
597 of aquatic insects. *Limnology and Oceanography* 19:154-156.

598 Statzner, B., and V.H. Resh. 1993. Multiple-site and -year analyses of stream insect emergence:
599 a test of ecological theory. *Oecologia* 96:65-79.

600 USDOE (United States Department of Energy). 2008a. Annual coal report. Energy Information
601 Administration, United States Department of Energy, Washington, DC.

602 USDOE (United States Department of Energy). 2008b. International energy annual report.

603 Energy Information Administration, United States Department of Energy, Washington,
604 DC.

605 USEPA (United States Environmental Protection Agency). 2005. Mountaintop Mining/Valley
606 Fills in Appalachia Final Programmatic Environmental Impact Statement. EPA Region
607 3, Philadelphia, PA. EPA 9-03-R-05002.
608 (<http://www.epa.gov/region3/mtntop/eis2005.htm>).

609 Wiley, J.B., R.D. Evaldi, J.H. Eychaner, and D.B. Chambers. 2001. Reconnaissance of stream
610 geomorphology, low streamflow, and stream temperature in the mountaintop coal-mining
611 region, West Virginia, United States Geological Survey, Water-Resources Investigations
612 Report 01-4092, Charleston, WV.

613 Woodcock, T.S. and A.D. Huryn. 2008. The effect of an interstate highway on
614 macroinvertebrate production in headwater streams. *Fundamental and Applied*
615 *Limnology* 171:199-218.

616 Woods, A.J., J.M. Omernik, D.D. Brown. 1999. Level III and IV Ecoregions of Delaware,
617 Maryland, Pennsylvania, Virginia, and West Virginia. United States Environmental
618 Protection Agency, Western Ecology Division, Corvallis, OR
619 (http://www.epa.gov/wed/pages/ecoregions/reg3_eco.htm)

620 Young, R.G., C.D. Matthaei, and C.R. Townsend. 2008. Organic matter breakdown and
621 ecosystem metabolism: functional indicators for assessing river ecosystem health.
622 *Journal of the North American Benthological Society* 27:605-625.

623 Xie, L., D.H. Funk, and D.B. Buchwalter. 2010. Trophic transfer of Cd from natural periphyton
624 to the grazing mayfly *Centroptilum triangulifer* in a life cycle test. *Environmental*
625 *Pollution* 158:272-277.

626 Zimmerman, G.M., H. Goetz, and P.W. Mielke Jr. 1985. Use of an improved statistical method
627 for group comparisons to study effects of prairie fire. *Ecology* 66: 606–611.
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Table 1. Watershed and stream channel characteristics for ten study streams in the Twentymile Creek watershed, WV. Categories are Forested (F), Mined Upstream of ponds (MU), and Mined downstream of ponds (MD). Upstream channel length represents total of main channel and all tributaries upstream based on National Hydrologic Database 1:24,000 scale maps.

| Stream | Category | Watershed Area (km ²) | Major Landuses (%) | | Channel Substrates (%) | | Upstream Channel Length (km) | Distance to Valley Fill (km) |
|-----------|----------|---|---------------------|--------|------------------------|--------------------|------------------------------------|------------------------------------|
| | | | Deciduous Forest | Barren | Mixed Substrates | Bedrock Outcrop | | |
| Ash | F | 3.99 | 100.0 | 0 | 95 | 5 | 5.89 | — |
| Jack | F | 0.91 | 99.7 | 0 | 80 | 20 | 1.26 | — |
| Laurel | F | 1.41 | 100.0 | 0 | 96 | 4 | 1.56 | — |
| Neil | F | 3.73 | 99.8 | 0.1 | 99 | 1 | 5.46 | — |
| Peters | F | 1.62 | 94.6 | 1.2 | 99 | 1 | 2.79 | — |
| Lost | MU | 0.79 | 8.1 | 91.9 | 97 | 3 | 1.06 | 0.16 |
| Beech | MU | 0.57 | 36.1 | 63.9 | 73 | 27 | 0.75 | 0.33 |
| Hardway | MD | 1.43 | 40.6 | 59.4 | 68 | 32 | 2.58 | 0.53 |
| Sugarcamp | MD | 5.27 | 18.9 | 77.4 | 97 | 3 | 8.73 | 1.40 |
| Buckles | MD | 2.61 | 61.9 | 38.1 | 98 | 2 | 3.71 | 0.97 |

Table 2. Stream discharge and physicochemistry for study streams in the Twentymile Creek Watershed, WV. Categories are Forested (F), Mined Upstream of ponds (MU), and Mined Downstream of ponds (MD). Temperature values are daily mean, others are mean of four seasonal measures (\pm S.E). Temperature and degree days cover entire study period (27 Sept. 07 to 14 July 08). GLIMPSS = Genus-level Index of Most Probable Stream Status from macroinvertebrate sampling, RBP = Rapid Bioassessment Protocol habitat score, and RCE = Riparian, Channel, and Environmental Inventory habitat score.

| Stream | Category | Discharge | Conductivity | pH | D.O. (mg/l) | Temp. (°C) | Accumulated |
|-------------------------|----------|---------------------|-----------------------|--------------------|---------------------|---------------------|-------------|
| | | (m ³ /s) | (μ S/cm) | | | | Degree Days |
| Ash | F | 0.076 (0.04) | 41.0 (4.5) | 6.20 (0.25) | 9.93 (1.09) | 9.88 (0.28) | 2866 |
| Jack | F | 0.013 (0.01) | 45.6 (1.2) | 6.71 (0.16) | 9.66 (1.51) | 10.26 (0.27) | 2980 |
| Laurel | F | 0.030 (0.02) | 41.6 (4.5) | 6.17 (0.19) | 10.00 (1.50) | 9.75 (0.26) | 2827 |
| Neil | F | 0.073 (0.04) | 49.3 (4.7) | 6.35 (0.25) | 10.41 (1.31) | 10.49 (0.27) | 2962 |
| Peters | F | 0.021 (0.01) | 81.9 (4.5) | 6.52 (0.13) | 10.06 (1.17) | 10.17 (0.28) | 3051 |
| Forested Average | | 0.043 (0.01) | 51.9 (7.7) | 6.39 (0.10) | 10.01 (0.12) | 10.11 (0.12) | 2937 |
| Lost | MU | 0.012 (0.002) | 2479.5 (45.9) | 7.73 (0.15) | 10.60 (0.85) | 11.30 (0.12) | 3297 |
| Beech | MU | 0.008 (0.001) | 1342.1 (99.3) | 7.17 (0.10) | 10.63 (1.52) | 10.66 (0.24) | 3105 |
| Hardway | MD | 0.024 (0.01) | 1706.7 (222.3) | 7.50 (0.01) | 10.25 (1.08) | 10.93 (0.31) | 3177 |
| Sugarcamp | MD | 0.073 (0.01) | 1452.6 (251.6) | 8.24 (0.29) | 9.05 (1.26) | 11.91 (0.37) | 3469 |
| Buckles | MD | 0.048 (0.01) | 1170.9 (74.6) | 7.94 (0.07) | 10.75 (0.99) | 10.48 (0.23) | 3063 |
| Mined Average | | 0.033 (0.01) | 1630.4 (229.4) | 7.72 (0.18) | 10.26 (0.31) | 11.06 (0.12) | 3222 |

Table 2 (cont.)

| GLIMPSS | RBP Total | RCE Total |
|----------------|------------------|------------------|
| 86.02 | 164 | 321 |
| 80.08 | 170 | 326 |
| 86.29 | 176 | 320 |
| 88.87 | 163 | 325 |
| 85.16 | 167 | 326 |
| 85.28 | 168.0 | 323.6 |
| 19.49 | 119 | 172 |
| 42.20 | 147 | 291 |
| 57.72 | 136 | 240 |
| 22.04 | 155 | 253 |
| 34.95 | 154 | 281 |
| 35.28 | 142.2 | 247.4 |

Table 3. Emergent trap recovery by stream, season, and habitat in headwater tributaries within the Twentymile Creek, WV watershed, Autumn 2007-Spring 2008. Categories F, MU, and MD as defined in Table 1.

| | | Autumn | | Winter | | Spring | | Summer | |
|------------------------|----|---------------|----------|---------------|----------|---------------|----------|---------------|----------|
| | | Riffle | Pool | Riffle | Pool | Riffle | Pool | Riffle | Pool |
| Category | | | | | | | | | |
| Ash | F | 1 | 0 | 2 | 1 | 2 | 1 | 0 | 0 |
| Jack | F | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 |
| Laurel | F | 2 | 1 | 2 | 1 | 2 | 1 | 1 | 0 |
| Neil | F | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 1 |
| Peters | F | 0 | 0 | 2 | 1 | 2 | 0 | 1 | 1 |
| Forested Totals | | 6 | 3 | 9 | 5 | 10 | 4 | 6 | 3 |
| Lost | MU | 2 | 0 | 2 | 1 | 2 | 1 | 0 | 0 |
| Beech | MU | 1 | 0 | 2 | 1 | 2 | 1 | 0 | 1 |
| Hardway | MD | 1 | 0 | 2 | 1 | 2 | 1 | 2 | 1 |
| Sugarcamp | MD | 2 | 1 | 1 | 1 | 2 | 1 | 2 | 1 |
| Buckles | MD | 2 | 1 | 2 | 1 | 2 | 1 | 0 | 0 |
| Mined Totals | | 8 | 2 | 9 | 5 | 10 | 5 | 4 | 3 |

Table 4. Statistical results for comparison of emergent aquatic insect density and biomass by treatment (mined versus forest) and season (autumn, winter, spring and summer), * indicates significant differences ($p < 0.05$).

| Dependent | | | | | | |
|---------------|---------------------|------|-------|------|-------|-----------------|
| Variable | Source of Variation | d.f. | SS | MS | F | <i>p</i> -value |
| Total Density | Full Model | 15 | 11.80 | 0.79 | 4.73 | 0.0008* |
| | Treatment | 1 | 0.32 | 0.32 | 1.91 | 0.182 |
| | Stream(Treatment) | 8 | 4.84 | 0.60 | 3.64 | 0.0091* |
| | Season | 3 | 5.62 | 1.87 | 11.27 | 0.0002* |
| | Season*Treatment | 3 | 0.36 | 0.12 | 0.73 | 0.55 |
| Total Biomass | Full Model | 15 | 10.74 | 0.72 | 3.91 | 0.0026* |
| | Treatment | 1 | 0.12 | 0.12 | 0.63 | 0.4374 |
| | Stream(Treatment) | 8 | 6.23 | 0.78 | 4.25 | 0.0041* |
| | Season | 3 | 3.69 | 1.23 | 6.71 | 0.0026* |
| | Season*Treatment | 3 | 0.21 | 0.07 | 0.38 | 0.77 |

Table 5. Statistical results for comparison of emergent density and biomass of combined Ephemeroptera, Plecoptera, and Trichoptera (EPT) by treatment and season. * indicates significant differences ($p < 0.05$).

| Dependent | | | | | | |
|-----------|---------------------|------|------|------|-------|-----------------|
| Variable | Source of Variation | d.f. | SS | MS | F | <i>p</i> -value |
| Total EPT | Full Model | 15 | 5.23 | 0.35 | 6.52 | <0.0001* |
| Density | | | | | | |
| | Treatment | 1 | 1.52 | 1.52 | 28.45 | <0.0001* |
| | Stream(Treatment) | 8 | 1.31 | 0.16 | 3.06 | 0.0202* |
| | Season | 3 | 1.34 | 0.45 | 8.37 | 0.0008* |
| | Season*Treatment | 3 | 0.33 | 0.11 | 2.07 | 0.137 |
| Total EPT | Full Model | 15 | 6.67 | 0.44 | 5.47 | 0.0003* |
| Biomass | | | | | | |
| | Treatment | 1 | 2.34 | 2.34 | 28.76 | <0.0001* |
| | Stream(Treatment) | 8 | 1.74 | 0.22 | 2.68 | 0.0354* |
| | Season | 3 | 1.49 | 0.49 | 6.09 | 0.0041* |
| | Season*Treatment | 3 | 0.49 | 0.16 | 2.02 | 0.1432 |

Appendix. Emergent density, Dens (individuals m⁻² d⁻¹), biomass, Biom (mg m⁻² d⁻¹), and estimated benthic production, Prod (mg AFDM m⁻² season/year⁻¹). Seasonal values are average of streams within treatment. Annual values for A and B are averages across seasons, whereas annual benthic P represent sum of seasonal values. Significant differences between treatments within A, B, or P categories for individual taxa/metric in bold.

| Taxon/Metric | Treatment | Season/Year | Dens | Biom | Prod |
|-------------------------|------------------|--------------------|--------------|-------------|----------------|
| Nematocera (Diptera) | Mined | Autumn | 35.53 | 9.28 | 4613.3 |
| | | Winter | 4.44 | 2.21 | 1084.6 |
| | | Spring | 25.17 | 14.08 | 7079.1 |
| | | Summer | 72.52 | 13.20 | 6634.2 |
| | | <i>Annual</i> | <i>34.41</i> | <i>9.69</i> | <i>19411.2</i> |
| | Forest | Autumn | 42.51 | 4.92 | 2445.2 |
| | | Winter | 1.90 | 0.43 | 209.2 |
| | | Spring | 27.35 | 3.05 | 1535.5 |
| | | Summer | 81.97 | 11.54 | 5801.4 |
| | | <i>Annual</i> | <i>38.43</i> | <i>4.98</i> | <i>9991.3</i> |
| Brachycera (Diptera) | Mined | Autumn | 0.29 | 0.06 | 30.8 |
| | | Winter | 0.01 | 0 | 0.3 |
| | | Spring | 0.07 | 0.09 | 44.8 |
| | | Summer | 2.23 | 0.22 | 111.1 |
| | | <i>Annual</i> | <i>0.65</i> | <i>0.09</i> | <i>187.0</i> |
| | Forest | Autumn | 0.73 | 0.07 | 36.0 |
| | | Winter | 0.05 | 0 | 1.8 |
| | | Spring | 0.19 | 0.04 | 18.6 |
| | | Summer | 0.36 | 0.04 | 18.2 |

| | | | | | |
|---------------|--------|---------------|-------------|-------------|---------------|
| | | <i>Annual</i> | <i>0.33</i> | <i>0.04</i> | <i>74.6</i> |
| Ephemeroptera | Mined | Autumn | 0.02 | 0.04 | 18.0 |
| | | Winter | 0 | 0 | 0 |
| | | Spring | 0.04 | 0.23 | 116.1 |
| | | Summer | 0 | 0 | 0 |
| | | <i>Annual</i> | <i>0.02</i> | <i>0.07</i> | <i>134.1</i> |
| | Forest | Autumn | 0.76 | 0.99 | 492.3 |
| | | Winter | 0 | 0 | 0 |
| | | Spring | 1.65 | 3.85 | 1933.4 |
| | | Summer | 4.82 | 3.93 | 1977.9 |
| | | <i>Annual</i> | <i>1.81</i> | <i>2.19</i> | <i>4403.7</i> |
| Plecoptera | Mined | Autumn | 0.08 | 0.41 | 202.5 |
| | | Winter | 0.62 | 0.60 | 295.5 |
| | | Spring | 0.10 | 0.62 | 309.7 |
| | | Summer | 0.05 | 0.17 | 83.7 |
| | | <i>Annual</i> | <i>0.21</i> | <i>0.45</i> | <i>891.4</i> |
| | Forest | Autumn | 0.43 | 2.28 | 1133.0 |
| | | Winter | 0.85 | 0.83 | 407.5 |
| | | Spring | 0.09 | 0.95 | 479.9 |
| | | Summer | 1.67 | 5.19 | 2610.4 |
| | | <i>Annual</i> | <i>0.76</i> | <i>2.31</i> | <i>4630.9</i> |
| Trichoptera | Mined | Autumn | 0.47 | 0.21 | 106.4 |
| | | Winter | 0 | 0 | 0 |

| | | | | | |
|-----------|--------|---------------|-------------|-------------|----------------|
| | | Spring | 0.02 | 0.03 | 15.4 |
| | | Summer | 0.91 | 0.84 | 420.7 |
| | | <i>Annual</i> | <i>0.35</i> | <i>0.27</i> | <i>542.5</i> |
| | Forest | Autumn | 0.12 | 0.14 | 71.8 |
| | | Winter | 0 | 0 | 0 |
| | | Spring | 0.22 | 0.26 | 131.7 |
| | | Summer | 3.13 | 2.22 | 1119.7 |
| | | <i>Annual</i> | <i>0.87</i> | <i>0.66</i> | <i>1323.3</i> |
| Odonata | Mined | Autumn | 0 | 0 | 0 |
| | | Winter | 0 | 0 | 0 |
| | | Spring | 0 | 0 | 0 |
| | | Summer | 0.06 | 20.02 | 10064.1 |
| | | <i>Annual</i> | <i>0</i> | <i>0</i> | <i>10064.1</i> |
| | Forest | Autumn | 0 | 0 | 0 |
| | | Winter | 0 | 0 | 0 |
| | | Spring | 0 | 0 | 0 |
| | | Summer | 0 | 0 | 0 |
| | | <i>Annual</i> | <i>0.01</i> | <i>5.00</i> | <i>0</i> |
| Total EPT | Mined | Autumn | 0.57 | 0.66 | 327.0 |
| | | Winter | 0.62 | 0.6 | 295.5 |
| | | Spring | 0.16 | 0.88 | 441.2 |
| | | Summer | 0.96 | 1.00 | 504.3 |
| | | <i>Annual</i> | <i>0.58</i> | <i>0.78</i> | <i>1567.9</i> |

| | | | | |
|--------|---------------|-------------|-------------|----------------|
| Forest | Autumn | 1.31 | 3.41 | 1697.2 |
| | Winter | 0.85 | 0.83 | 407.5 |
| | Spring | 1.97 | 5.06 | 2545.1 |
| | Summer | 9.62 | 11.35 | 5708.1 |
| | <i>Annual</i> | <i>3.44</i> | <i>5.16</i> | <i>10357.9</i> |

List of Figures

Figure 1. Seasonal emergent aquatic A) density (indiv. $\text{m}^{-2} \text{d}^{-1}$) and B) biomass ($\text{mg m}^{-2} \text{d}^{-1}$) (+/- S.E.) from mined (n=5) and forested (n=5) subcatchments of the Twentymile Creek, WV watershed, Autumn 2007-Spring 2008.

Figure 2. Estimated annual secondary production ($\text{g AFDM m}^{-2} \text{y}^{-1}$) with relative taxonomic contribution for mined and forested streams in the Twentymile Creek, WV watershed, Autumn 2007-Spring 2008.



