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# Temporal changes in taxonomic and functional diversity of fish assemblages downstream from mountaintop mining

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**Abstract:** Mountaintop mining (MTM) affects chemical, physical, and hydrological properties of receiving streams, but the long-term consequences for fish-assemblage structure and function are poorly understood. We sampled stream fish assemblages using electrofishing techniques in MTM exposure sites and reference sites within the Guyandotte River basin, USA, during 2010–2011. We calculated indices of taxonomic diversity (species richness, abundance, Shannon diversity) and functional diversity (functional richness, functional evenness, functional divergence) to compare exposure and reference assemblages between seasons (spring and autumn) and across years (1999–2011). We based temporal comparisons on 2 sites that were sampled during 1999–2001 by Stauffer and Ferreri (2002). Exposure assemblages had lower taxonomic and functional diversity than reference assemblages or simulated assemblages that accounted for random variation. Differences in taxonomic composition between reference and exposure assemblages were associated with conductivity and aqueous Se concentrations. Exposure assemblages had fewer species, lower abundances, and less biomass than reference assemblages across years and seasons. Green Sunfish (*Lepomis cyanellus*) and Creek Chub (*Semotilus atromaculatus*) became numerically dominant in exposure assemblages over time because of their persistence and losses of other taxa. In contrast, species richness increased over time in reference assemblages, a result that may indicate recovery from drought. Mean individual biomass increased as fish density decreased and most obligate invertivores were apparently extirpated at MTM exposure sites. Effects of MTM were not related to physical-habitat conditions but were associated with water-quality variables, which may limit quality and availability of benthic macroinvertebrate prey. Simulations revealed effects of MTM that could not be attributed to random variation in fish assemblage structure.

**Key words:** fish community, species richness, functional diversity, simulation, mining

Mountaintop mining (MTM) is a surface-mining technique that exposes coal seams for extraction by disposing of overlying soil and rock layers in adjacent valleys (valley fills). MTM requires construction of valley fills and may lower mountaintop elevations by as much as 300 m (USEPA 2011b). This mining method has been used in the central Appalachian region of eastern North America (West Virginia, Virginia, Kentucky) since the 1960s, but its spatial extent has greatly expanded over the last 20 y (USEPA 2011b). Over 2000 fluvial km (fkm) of headwater streams have been buried by MTM valley fills in this region (Bernhardt and Palmer 2011), and additional mining permits have been issued (USEPA 2011b). MTM is the primary driver of landuse change in central Appalachia (Bernhardt and Palmer 2011).

Downstream ecosystems are influenced by MTM through hydrological, physical, and chemical pathways. Base flows

are typically increased downstream from valley fills because of decreased evapotranspiration and increased imperviousness of MTM headwater areas (Wiley et al. 2001, Messinger 2003). Erosional processes from valley fills may cause decreased substrate particle size in downstream reaches (Paybins et al. 2000, Wiley et al. 2001) and increased stream conductivity and dissolved metal concentrations (Griffith et al. 2012). Se discharge and bioaccumulation in downstream ecosystems is a particular concern (Palmer et al. 2010, Presser and Luoma 2010, Lindberg et al. 2011). Benthic macroinvertebrate assemblages consistently lose mayfly taxa (Ephemeroptera) downstream of valley fills (Chambers and Messinger 2001, Hartman et al. 2005, Pond et al. 2008, Pond 2010), probably because of osmoregulatory stress caused by increased ionic strength of surface waters (McCulloch et al. 1993).

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Few investigators have evaluated effects of MTM on stream fishes. Stauffer and Ferreri (2002) sampled stream fish assemblages in southern West Virginia and eastern Kentucky and found that fish assemblages downstream of valley fills supported  $\sim\frac{1}{2}$  the number of species found in reference sites. They suggested that Se contamination was a primary cause of loss of fish richness (Stauffer and Ferreri 2002). Fulk et al. (2003) calculated multimetric bioassessment scores from the Stauffer and Ferreri (2002) data set and reported decreased cyprinid richness, decreased benthic invertivore richness, and increased proportions of tolerant individuals in small watersheds (2–10 km<sup>2</sup>) downstream of MTM. Confounding effects of residential development can obscure analysis of MTM in larger streams (Fulk et al. 2003, Merriam et al. 2011). McGarvey and Johnston (2013) used energetic models and mining permit data to estimate potential effects of MTM on Smallmouth Bass (*Micropterus dolomieu*) and Brook Trout (*Salvelinus fontinalis*) and estimated  $\sim 12$  to 14% decreases in their abundances in the Coal River basin, West Virginia. Upstream MTM mean patch size was negatively associated with occurrence probabilities for 4 of 6 fish species evaluated by Hopkins and Roush (2013). The assertion by the USEPA (2011b) that “fish communities are consistently degraded” downstream of MTM is based primarily on the data set by Stauffer and Ferreri (2002).

Temporal responses of fish assemblages to headwater MTM have not been studied. Fish assemblages are temporally variable in response to stressors because of movement of individuals to access remote resources, escape unsuitable habitats, and recolonize extirpated populations (Schlosser 1991). Such movement patterns can affect fish assemblage composition and bioassessments of stream networks (Hitt and Angermeier 2008a, b) and, therefore, could affect fish assemblage responses to MTM. A temporal perspective is particularly important in this case because Stauffer and Ferreri’s (2002) collections included years of extreme drought in West Virginia (USEPA 2005), and droughts reduce local fish species richness and abundance via emigration and extirpation (Larimore et al. 1959, Matthews and Marsh-Matthews 2003, Adams and Warren 2005). We resampled a subset of sites sampled by Stauffer and Ferreri (2002) to evaluate effects of MTM over time and to explore the prediction that responses resulting from drought recovery would be more evident at reference than exposure sites.

We evaluated potential effects of MTM on fish functional and taxonomic diversity. Functional diversity is represented by the composition of species traits and life-history strategies in ecological assemblages (Petchey and Gaston 2006) and typically is quantified from attributes representing species traits and abundance in multidimensional ordination space (Cornwell et al. 2006, Mason et al. 2005, Villéger et al. 2008). Spatial and temporal variation in functional di-

versity may indicate the relative importance of environmental and competitive mechanisms of community assembly (Mason et al. 2012) and can inform analysis of assemblage responses to environmental quality (Villéger et al. 2010, Luck et al. 2013). Such approaches offer advantages over taxonomic or single-trait analyses because natural selection operates on many interacting traits simultaneously. Moreover, zoogeography may confound taxonomic variation but is of less concern for species traits (Poff 1997, Verberk et al. 2013).

## METHODS

We evaluated stream fish assemblages within the Guyandotte River basin, West Virginia, USA (Fig. 1). The Guyandotte River basin encompasses 4350 km<sup>2</sup> within the Allegheny Plateau region of southwestern West Virginia. The mainstem Guyandotte River flows for  $\sim 270$  km before its confluence with the Ohio River near Huntington, West Virginia. Surficial geology of the basin is dominated by Pennsylvanian-aged sandstone/shale valley bottoms and shale/siltstone ridges (Nicholson et al. 2007). The study area lacks extensively urbanized areas and land use consists primarily of forestry and coal-mining operations (USEPA 2005). We restricted our analysis to the Guyandotte River basin to control for zoogeographic and physiographic influences on fish assemblage structure (Hitt and Angermeier 2011).

We sampled in the Mud River watershed (930 km<sup>2</sup>), which contains the largest tributary of the Guyandotte River (Fig. 1). This watershed has extensive coal-mining operations and has been the subject of research on the effects of mining on flow regimes (Wiley et al. 2001), water quality (Lindberg et al. 2011), and Se bioaccumulation (Presser and Luoma 2010, WVDEP 2010). Over 100 point-source pollution-discharge permits associated with surface mining have been issued in the upper Mud River watershed (Lindberg et al. 2011).

We evaluated temporal and seasonal changes in fish assemblage composition in different pairs of sites (temporal exposure [TE] vs temporal reference [TR] and seasonal exposure [SE] vs seasonal reference [SR]). In 2011, we resampled an MTM exposure site that was originally sampled in 1999, 2000, and 2001 (Mud River near Connelly’s Branch, Table 1; site TE in Fig. 1) and a reference site that was originally sampled in 2001 (Laurel Creek, Table 1; site TR in Fig. 1). These sites were used to analyze longer-term temporal changes. The original samples from 1999–2001 were collected for an Environmental Impact Statement (USEPA 2005) by Stauffer and Ferreri (2002). Our sampling methods closely replicated the original methods. We blocknetted reaches and collected fishes with 3-pass electrofishing with 2 backpacks at the exposure site and 1 backpack at the reference site (Table 1). We separated fishes by pass number and preserved them in 10% buffered

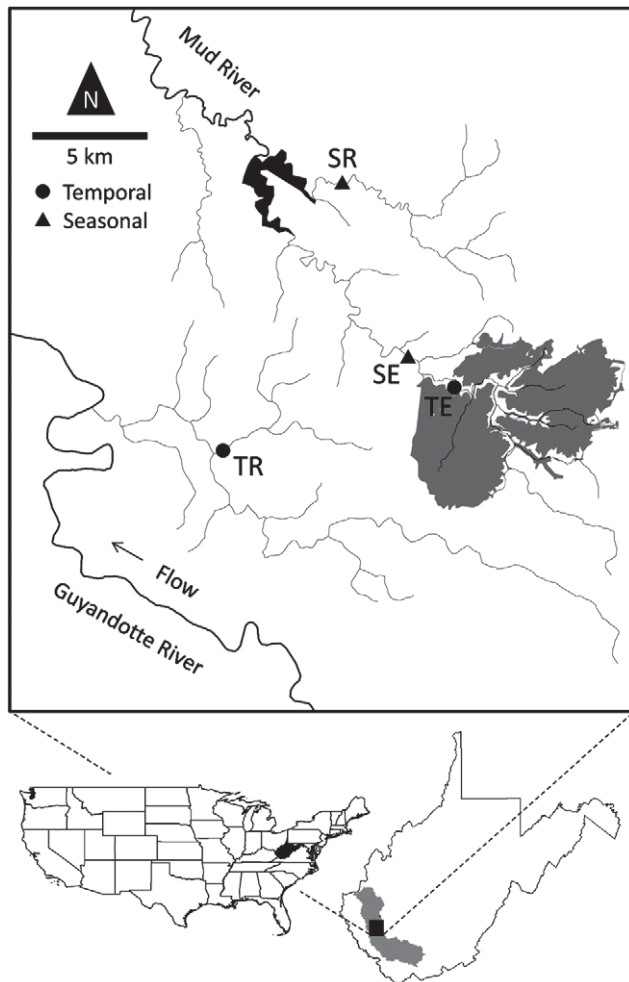


Figure 1. Sampling sites in the Guyandotte River basin (inset), West Virginia. Mountaintop mining exposure sites were in the Mud River near Connelly's Branch (temporal exposure = TE) and Berry Branch (seasonal exposure = SE). Reference sites were in Laurel Creek (temporal reference = TR) and the Left Fork of the Mud River (seasonal reference = SR). Gray polygon shows the approximate mining permit boundary (2010 data).

formalin. In the laboratory, we identified all individuals to species and weighed individuals to nearest 0.1 g. We calculated total biomass as the sum of masses of all fish caught at each site (Stauffer and Ferreri 2002).

We supplemented temporal data with seasonal samples collected from an additional exposure site and reference site during autumn 2010 and spring 2011 (Table 1). The seasonal-exposure site was in the Mud River near Berry Branch (site SE in Fig. 1), and the seasonal-reference site was on the Left Fork of the Mud River (site SR in Fig. 1) (Table 1). For these samples, we used 1-pass backpack electrofishing and dipnets (2 concurrent backpack units). We sampled reach lengths that were 40× the mean stream width (Lazorchak et al. 2000) (Table 1). Fishes were

identified in the field, counted, and released (voucher specimens were retained for some *Notropis* species). All fish samples were collected during baseflow conditions. For comparisons among years, seasons, and landuse treatments, we scaled species richness, abundance, and biomass data to equivalent units of sampled stream area (100 m<sup>2</sup>).

The period of analysis (1999–2011) encompassed a substantial increase in upstream MTM operations. The extent of surface mining in the Mud River watershed increased from ~8% in 1995 to 33% in 2005 (Amos and Campagna 2011). Mining activities also moved closer to exposure sites during this period because of MTM and valley fill construction in Connelly's Branch, <5 km upstream of exposure sites (Fig. 1). Therefore, our temporal analysis may indicate fish assemblage responses to chronic and increasing MTM exposure over a 12-y period but cannot resolve the total effects of headwater mining.

Fish assemblage samples in 2010 and 2011 were collected during nondrought periods (NDMC 2013), whereas the sampling frame of Stauffer and Ferreri (2002) included exceptional droughts in the southeastern USA (Adams and Warren 2005) and state and federally declared droughts in West Virginia (USEPA 2005). Annual flows in the Guyandotte River in 1999–2001 were 5.1 to 12.2 m<sup>3</sup>/s below long-term mean annual flows (1963–2011), whereas annual flows in 2010–2011 were within 0.7 m<sup>3</sup>/s of the long-term annual mean for this gage station (Guyandotte River at Logan, West Virginia; US Geological Survey [USGS] gage 03203600).

We used nonmetric multidimensional scaling (NMDS) to compare fish assemblage composition between exposure and reference sites (all sites and sampling events combined). We used Bray–Curtis distances of species abundance standardized by sampling area, and we did not exclude rare taxa. Differences in assemblage composition were assessed with 95% confidence ellipses calculated from the standard deviation of species scores in 2-dimensional space. We evaluated water-quality covariates by fitting general linear model trend surfaces for stream conductivity and total dissolved Se concentrations (Table 1). We estimated conductivity and Se concentrations in unsampled sites from mean values in observed exposure and reference samples (Table 1). We used a total dissolved Se value of 0.5 µg/L when water samples were below detection limits (1 µg/L; May et al. 2007). Ordination techniques were implemented with R package *vegan* (version 2.0-7; Oksanen et al. 2013).

For analysis of functional diversity, we characterized species traits based on body size, reproductive strategy, and trophic strategy. We indexed body size as the maximum reported total length (TL). Reproductive variables included maximum fecundity, age of female maturation, and longevity. We also classified species as belonging to 1 of 3 spawning types: simple lithophils (spawning over open rock and gravel substrate and lacking parental care), brood hidiers (spawning

Table 1. Site characteristics and sampling methods. Data from 1999–2001 were collected by Stauffer and Ferreri (2002). ND = no data collected.

Treatment	Site name (code)	Upstream basin area (km <sup>2</sup> )	Electrofishing methods		Water quality	
			Sampling date	Backpack count, pass count, sampling area (m <sup>2</sup> )	Conductivity (μS/cm)	Dissolved Se (μg/L) <sup>a</sup>
Temporal						
Exposure	Mud River near Connelly's Branch (TE)	39.4	25 October 1999	2, 3, 574	1440	ND
			1 April 2000	2, 3, 668	686	13.0 <sup>b</sup>
			14 September 2001	2, 3, 700	1836	12.1
			25 September 2011	2, 3, 708	1750	ND
Reference	Laurel Creek (TR)	24.5	15 September 2001	1, 3, 341	125	Nondetect
			26 October 2011	1, 3, 370	96	ND
Seasonal						
Exposure	Mud River near Berry Branch (SE)	66.7	30 September 2010	2, 1, 1000	1900	12.7 <sup>c</sup>
			2 May 2011	2, 1, 1000	ND	18.2 <sup>d</sup>
Reference	Left Fork of Mud River (SR)	35.0	30 September 2010	2, 1, 640	170	Nondetect <sup>e</sup>
			9 May 2011	2, 1, 640	ND	Nondetect <sup>d</sup>

<sup>a</sup> 2000–2001 samples analyzed by Research Environmental and Industrial Consultants, Inc.; 2010–2011 samples analyzed by US Geological Survey Columbia Environmental Research Center

<sup>b</sup> Sampled on 30 August 2000

<sup>c</sup> Sampled 10 November 2010

<sup>d</sup> Sampled 1 June 2011

<sup>e</sup> Sampled 8 November 2010

below substrate surface and lacking parental care), and nest-guarders (establishing and defending a territory within a spawning nest or cavity). We based spawning types on categories by Balon (1975) as synthesized by Simon (1999). We classified fish species into 1 of 3 feeding-strategy types: omnivore–herbivores, obligate invertivores, and invertivore–piscivores. We assembled species traits data from works by Frimpong and Angermeier (2009) and Hitt and Angermeier (2008b, 2011) (Appendix S1).

We quantified attributes of functional diversity as functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv) (Mason et al. 2005, Villéger et al. 2008). FRic represents the diversity of species trait combinations in an assemblage as indexed by the multidimensional volume of species traits (i.e., convex hull) in principal coordinates analysis. FEve measures the evenness of species abundances in the multidimensional space defined by FRic, and FDiv measures the divergence of species in this space with respect to their abundance and proximity to the extreme trait-combinations (i.e., convex hull boundary) (Mason et al. 2005, Villéger et al. 2008). Functional diversity calculations were implemented with R package *FD* (version 1.0-11; Laliberté and Shipley 2011).

To evaluate the significance of observed differences between MTM treatments, we compared observed differences in taxonomic and functional indices to the distri-

bution of differences calculated from simulated null assemblages that lacked temporal variation in abundance (Mason et al. 2012). We generated simulated assemblages from observed pairwise samples wherein abundances for each species had an equal probability of being represented by either the 1<sup>st</sup> or 2<sup>nd</sup> observed value (i.e., matrix-swap null model). We calculated standardized effect sizes (SES) as:

$$SES = \frac{\Delta I_{obs} - \Delta X_{sim}}{sd_{sim}} \quad (\text{Eq. 1})$$

where  $\Delta I_{obs}$  is the observed pairwise difference in the index value between time periods, seasons, or landuse treatments,  $\Delta X_{sim}$  is the mean of differences in functional index values for simulated assemblages, and  $sd_{sim}$  is the standard deviation of the differences in index values for simulated assemblages (Villéger et al. 2008, Mason et al. 2012). We used 10,000 replicates for null assemblage simulations in each pairwise combination of year, season, and landuse treatment. We assessed significance from the proportion of the simulated assemblage comparisons that were more extreme than the observed differences (i.e., type I error rate). Null assemblage simulations were implemented in

R (version 2.15.1; R Project for Statistical Computing, Vienna, Austria).

To assess potential differences in stream habitat between exposure and reference sites, we sampled substrate composition, thalweg depths, riparian canopy cover, and riffle, pool, and run availability at SE and SR during base-flow conditions in 2010. We quantified substrate size by classifying 100 randomly selected particles among size categories (Wentworth scale) as described by Wolman (1954). We measured thalweg depths and mesohabitat classes (pool, riffle, run) at 100 evenly spaced locations in the thalweg from the downstream to upstream limits of each sample reach. We measured canopy cover with a spherical densiometer at cardinal directions for 11 evenly spaced transects along the sampling reach (44 observations/site). These methods are commonly used to assess stream physical habitat in the Appalachian mountain region (Lazorchak et al. 2000). We could not assess temporal change in physical habitat data at TE and TR because data were not collected during the original sampling period.

## RESULTS

The combined fish data set included 2669 individuals representing 34 species and 6 families (Appendix S2). Cyprinid, percid, and centrarchid taxa were numerically dominant, and constituted 82% of total species richness and 94% of total abundance. In contrast, suckers, catfishes, and lampreys cumulatively contributed only 6 species (18%) and 150 individuals (6%) to the combined data set. Total fish biomass was dominated by cyprinids (49%), centrarchids (28%), and catostomids (15%) (Appendix S2). Creek Chub (*Semotilus atromaculatus*) had the greatest abundance and biomass among taxa in the combined data set (19% and 14%, respectively; Appendix S2).

A 2-dimensional NMDS ordination indicated differences in fish assemblage composition between exposure and reference sites as defined by 95% confidence ellipses (Fig. 2A, B). Linear trend surface analysis identified stream conductivity (Fig. 2A) and Se concentration (Fig. 2B) gradients associated with the transition from reference to exposure assemblages. Between 2001 and 2011, TE in 2001 differed more from TE in 2011 than TR in 2001 differed from TR in 2011 in NMDS space (i.e., more change in assemblage composition; Fig. 2A, B). The 95% confidence ellipse intersection of reference and exposure assemblages was associated with conductivities between ~600 to 1000  $\mu\text{S}/\text{cm}$  (Fig. 2A) and total dissolved Se concentrations between ~4 to 7  $\mu\text{g}/\text{L}$  (Fig. 2B). Thalweg depths were greater in SE than SR (Wilcoxon rank-sum test,  $p < 0.001$ ; Fig. 3A). SE and SR did not differ in substrate size distributions ( $\chi^2 = 7.8$ ,  $p = 0.16$ ; Fig. 3B); pool, riffle, and run habitat availability ( $\chi^2 = 3.6$ ,  $p = 0.16$ ; Fig. 3C); or riparian canopy coverage (Wilcoxon rank-sum test,  $p = 0.79$ ; Fig. 3D).

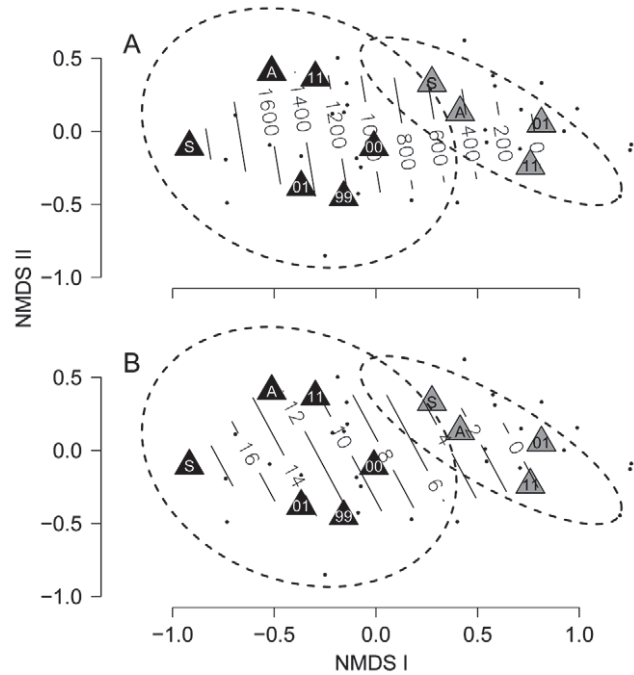


Figure 2. Nonmetric multidimensional scaling (NMDS) ordination of stream fish assemblage composition in mountaintop mining exposure and reference sites. Sites (triangles) are coded by sampling year (99 = 1999, 00 = 2000, 01 = 2001, 11 = 2011) or season (spring [S] or autumn [A]). Gray indicates reference sites, and black indicates exposure sites. Points indicate species. Dashed lines indicate 95% confidence ellipses for exposure and reference sites. Linear trend surfaces were fit from general additive models for stream conductivity ( $\mu\text{S}/\text{cm}$ ) (A) and total dissolved Se concentration ( $\mu\text{g}/\text{L}$ ) (B).

Taxonomic diversity differed between reference and exposure sites. Across all samples, reference sites supported an average of 2.3 more species/100  $\text{m}^2$  than exposure sites (4.3 vs 2.0 species/100  $\text{m}^2$ , respectively) and 58 more individuals (ind)/100  $\text{m}^2$  than exposure sites (91 vs 33 ind/100  $\text{m}^2$ , respectively; Table 2). Over the study period (1999–2011), species richness at TE decreased 49% (3.5 to 1.8 species/100  $\text{m}^2$ ), total abundance decreased 80% (89.1 to 17.4 ind/100  $\text{m}^2$ ), and Shannon diversity decreased 24% (2.1 to 1.6) (Table 2). Observed taxonomic changes in TE over the study period differed from null assemblage simulations (Table 3). Year-wise comparisons indicated that taxonomic changes were not evenly distributed over time. Species richness and abundance decreased at TE between 1999 and 2001 but were stable between 2001 and 2011 (Table 3). In contrast, species richness at TR increased between 2001 and 2011 from 5.0 to 6.2 species/100  $\text{m}^2$  (Table 2), but abundance and Shannon diversity did not differ between 2001 and 2011 (Table 3).

Taxonomic changes were associated with apparent extirpations at TE and colonization events at TR. Four cyp-

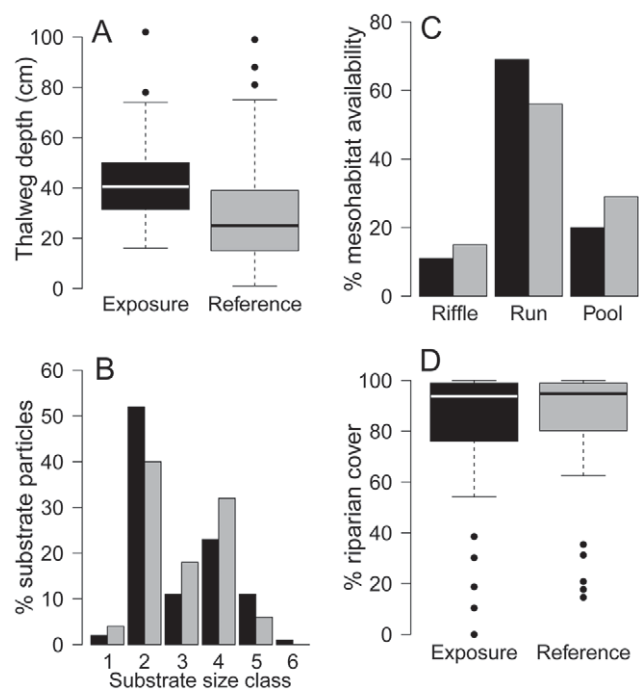


Figure 3. Thalweg depth (A), substrate size distribution (B), % pool, riffle, and run availability (C), and % riparian cover (D) at seasonal mountaintop mining exposure and reference sites (Sites SE and SR, Fig. 1). Substrate size categories were: 1) clay/silt, 2) sand, 3) fine gravel, 4) coarse gravel, 5) cobble, and 6) boulder. Box-and-whisker plots show medians (lines in boxes), quartiles (box ends), 95<sup>th</sup> percentiles (whiskers), and outliers (dots).

rinid species, 3 centrarchid species, and 3 percid species were detected before 2011 but not in 2011 at TE (i.e., apparent extirpations): Silverjaw Minnow (*Notropis buccatus*), Silver Shiner (*Notropis photogenis*), Rosyface Shiner (*Notropis rubellus*), Bluntnose Minnow (*Pimephales notatus*), Rock Bass (*Ambloplites rupestris*), Spotted Bass (*Micropterus punctulatus*), Largemouth Bass (*Micropterus salmoides*), Johnny Darter (*Etheostoma nigrum*), Banded Darter (*Etheostoma zonale*), and Blackside Darter (*Percina maculata*) (Appendix S2). One species (Least Brook Lamprey [*Lampetra aepyptera*]) was detected for the first time in 2011, indicating a possible colonization event at TE (Appendix S2). In contrast, 7 species were detected at TR during 2011 but not 2001 (i.e., apparent colonizations): Spottail Shiner (*Cyprinella spiloptera*), Spottail Shiner (*Notropis hudsonius*), *N. rubellus*, White Sucker (*Catostomus commersonii*), Golden Redhorse (*Moxostoma erythrurum*), Bluegill (*Lepomis macrochirus*), and *M. punctulatus*. Of the 24 species cumulatively observed at TR, only 1 species (Blacknose Dace [*Rhinichthys atratulus*]) occurred prior to but not during 2011 (Appendix S2).

Total fish biomass increased at TE and TR between 2001 and 2011, but total biomass at TE was <1/2 of total biomass at TR in both years (Fig. 4A). Total biomass at TE decreased between 1999 and 2001 but returned to near-1999 levels in 2011 (Fig. 4A). Mean individual biomass increased at TR and TE between 2001 and 2011, but individual biomass at TE far exceeded that at TR in both years (Fig. 4B). Mean individual biomass increased monotonically from 1999 to 2011 at TE (Fig. 4B).

Table 2. Taxonomic and functional diversity in mountaintop mining exposure and reference sites. Taxonomic diversity includes species richness (S), total abundance (N), and Shannon diversity (H'). Functional diversity includes functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv). Sites are mapped in Fig. 1.

Treatment (site code)	Year or season	Taxonomic			Functional		
		S <sup>a</sup>	N <sup>b</sup>	H'	FRic	FEve	FDiv
Temporal <sup>a</sup>							
Exposure (TE)	1999	3.5	89.1	2.1	1.87	0.46	0.89
	2000	3.0	46.9	2.4	1.15	0.61	0.85
	2001	1.7	15.3	2.0	0.05	0.45	0.87
	2011	1.8	17.4	1.6	0.17	0.71	0.97
Reference (TR)	2001	5.0	154.0	2.3	0.66	0.52	0.79
	2011	6.2	140.8	2.0	3.51	0.50	0.66
Seasonal <sup>b</sup>							
Exposure (SE)	Autumn	0.9	6.8	1.5	0.16	0.68	0.95
	Spring	0.8	4.9	1.5	<0.01	0.57	0.77
Reference (SR)	Autumn	3.3	52.8	2.3	1.29	0.66	0.88
	Spring	2.8	17.8	2.2	2.26	0.63	0.83

<sup>a</sup> Sites A and B, Fig. 1

<sup>b</sup> Sites C and D, Fig. 1

Table 3. Temporal and seasonal contrasts in taxonomic and functional diversity of stream fish assemblages in mountaintop mining exposure (Exp) and reference (Ref) sites. Cells show differences in species richness (S), total abundance (N), Shannon diversity (H'), functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv) between years, seasons, and landuse treatments. Values are expressed as standardized effect size (SES). Negative values indicate decreases over time or lower values in Exp samples than Ref samples. \* =  $p < 0.05$ , \*\* =  $p < 0.005$ . Sites are mapped in Fig. 1.

Treatment (site code)	Contrast	Taxonomic			Functional		
		S	N	H'	FRic	FEve	FDiv
<b>Temporal</b>							
Exp (TE)	2000–1999	<0.01	-1.92*	1.73*	-1.04	1.55	-0.74
	2001–2000	-3.56**	-3.44**	-2.32*	-2.84**	-0.16	0.93
	2001–1999	-3.98**	-3.46**	-0.40	-3.25**	1.70*	0.18
	2011–2001	0.46	0.38	-1.23	0.32	0.81	1.02
	2011–2000	-2.99**	-2.38*	-3.68**	-1.00	0.97	2.25*
	2011–1999	-2.99**	-2.86**	-2.21*	-1.64	2.01*	1.53
Ref (TR)	2011–2001	2.98**	-0.02	-1.44	3.02**	-0.44	-1.25
Exp vs Ref	2001 (Exp)–2001 (Ref)	-1.72*	-3.21**	-1.02	-1.18	0.42	1.21
	2011 (Exp)–2011 (Ref)	-3.35**	-2.11*	-0.89	-3.43**	1.94*	1.82*
<b>Seasonal</b>							
Exp (SE)	Spring–Autumn	-0.63	-1.19	0.45	-0.04	-0.70	-1.65
Ref (SR)	Spring–Autumn	-1.93*	-3.57**	0.23	2.15*	-0.80	-0.76
Exp vs Ref	Autumn (Exp)–Autumn (Ref)	-4.50**	-9.41**	-2.10*	-5.89**	0.16	1.31
	Spring (Exp)–Spring (Ref)	-3.31**	-0.14	-2.20*	-3.91**	-0.05	-0.18

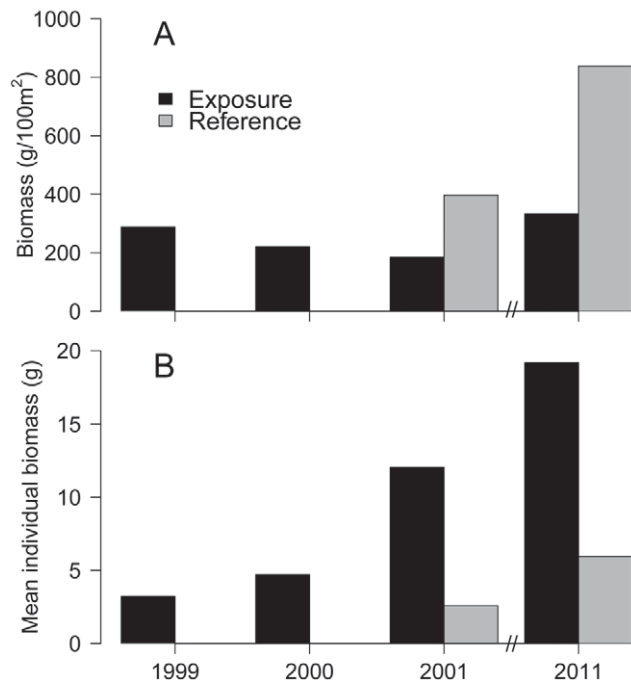


Figure 4. Temporal variation in stream fish total biomass (A) and mean biomass/individual (ind) (B) in mountaintop mining temporal exposure and reference sites (sites TE and TR; Fig. 1).

Functional diversity differed between exposure and reference sites, and the differences increased over time (Table 3). In 2011, TE had lower FRic and higher FEve and FDiv than TR, but TE and TR did not differ in this regard in 2001 (Table 3). Functional diversity indices did not differ between seasons at SE (Table 3), but FRic increased at SR between autumn and spring collections (Table 3). FRic was higher at SR than at SE, but FEve and FDiv did not differ between SR and SE (Table 3).

Changes in functional diversity reflected changes in species abundances and their associated trophic and reproductive strategies (Fig. 5A–C). Green Sunfish (*Lepomis cyanellus*) and *S. atromaculatus* became numerically dominant in TE over time and increased from 19% of fish abundance in 1999 to 75% in 2011 (Fig. 5A). This increased percentage arose from their persistence coupled with losses of other taxa. For instance, 96 *L. cyanellus* and *S. atromaculatus* were detected in 1999 and 92 were detected in 2011, and total fish abundance in this site decreased from 511 individuals in 1999 to 123 individuals in 2011 (Appendix S2).

Taxonomic changes at TE were associated with increasing dominance of invertivore–piscivore trophic strategies and decreasing relative abundance of omnivore–herbivores and obligate invertivores (Fig. 5B). For instance, obligate invertivores constituted ~50% of total fish abundance at TE in 1999, 2000, and 2001 but decreased to ~15% in 2011. In contrast, TR had >50% obligate invertivores in both periods



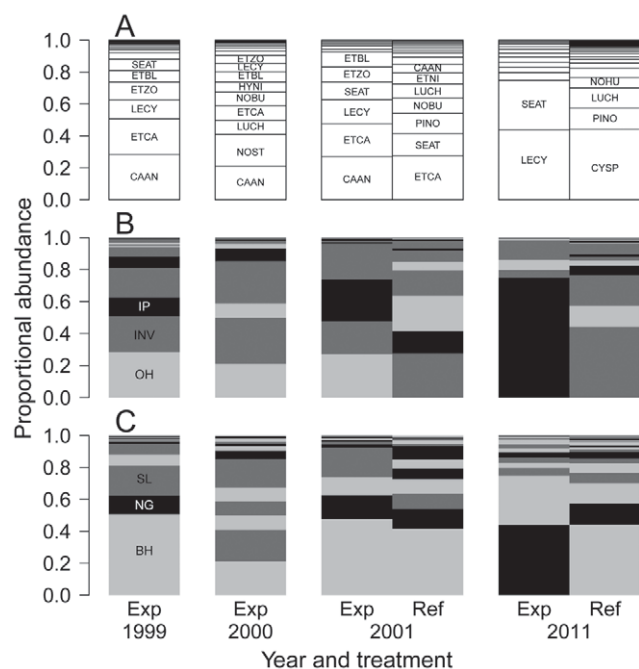


Figure 5. Temporal variation in species proportional abundances (A), trophic traits (B), and reproductive traits (C) in mountaintop mining exposure (Exp) and reference sites (Ref) (sites TE and TR, Fig. 1). OH = omnivore–herbivores, INV = obligate invertivores, IP = invertivore–piscivores, BH = brood hiders, SL = simple lithophils, NG = nest guardians. Species codes are given for *Campostoma anomalum* (CAAN), *Cyprinella spiloptera* (CYSP), *Etheostoma blennioides* (ETBL), *Etheostoma caeruleum* (ETCA), *Etheostoma nigrum* (ETNI), *Etheostoma zonale* (ETZO), *Hypentelium nigricans* (HYNI), *Lepomis cyanellus* (LECY), *Luxilus chrysocephalus* (LUCH), *Notropis buccatus* (NOBU), *Notropis hudsonius* (NOHU), *Notropis stramineus* (NOST), *Pimephales notatus* (PINO), and *Semotilus atromaculatus* (SEAT). Appendix S1 provides species traits data.

(Fig. 5B). Reproductive strategies at TE generally shifted toward nest-guarding and away from simple lithophilic spawning strategies over time (Fig. 5C). In contrast, fishes with brood-hiding reproductive strategies were most abundant at TR in both periods, even though the contributing species switched from Rainbow Darter (*Etheostoma caeruleum*) and *S. atromaculatus* in 2001 to *C. spiloptera* in 2011 (Fig. 5C).

Seasonal variation was greater at SR than at SE. SR supported fewer species, lower abundances, and higher FRic in spring than in autumn (Table 2), and all comparisons differed from null simulations (Table 3). In contrast, taxonomic and functional diversity indices at SE did not differ between spring and autumn (Table 3). Seasonal variation could not account for the observed differences between landuse treatments because SES values for exposure and reference site comparisons exceeded SES values for seasonal comparisons (Table 3).

## DISCUSSION

The central Appalachian mountains of North America are a global hotspot of freshwater fish biodiversity (Lévêque et al. 2008), and MTM is the primary source of landuse change in this region (Bernhardt and Palmer 2011). Prior studies indicated significant losses of species richness (Stauffer and Ferreri 2002) and diminished occurrence probabilities for some fish species downstream of MTM operations (Hopkins and Roush 2013). Our study expands the scope of prior research by addressing effects of MTM on taxonomic and functional diversity of stream fish assemblages over time. Our use of null assemblage simulations revealed effects of MTM that could not be attributed to random variation in fish assemblage composition.

Water quality and fish assemblages were consistent with expectations for high- and low-quality streams in the Appalachian region. Se concentrations and conductivities (Table 1) were within the range of values reported previously in the study area (Lindberg et al. 2011). Richness at exposure sites was below the interquartile range for Wadeable streams in the Ohio River zoogeographic region, but within the expected range at reference sites (Hitt and Angermeier 2011). Observed decreases in Shannon diversity at TE constituted a shift towards extreme conditions observed elsewhere in the Appalachian region. For example, in 1999, Shannon diversity at TE was 2.1, a value greater than values for 57% of the stream fish assemblages sampled in a probabilistic survey in West Virginia (Detenbeck and Cincotta 2008). However, in 2011, Shannon diversity at TE was 1.6, a value greater than only 23% of the samples collected by Detenbeck and Cincotta (2008). Increasing assemblage homogenization resulting from the persistence of *L. cyanellus* and *S. atromaculatus* was not surprising given the known tolerance of these species to environmental degradation (Karr 1981, Lemly 1985, Leonard and Orth 1986).

MTM also affected functional diversity of fish assemblages. We found significant losses of FRic downstream from MTM (Table 3) and similar patterns have been observed in estuarine fish assemblages responding to habitat degradation and eutrophication (Villéger et al. 2010) and plant assemblages responding to agricultural and urban development (Pakeman 2011). Loss of FRic suggests that MTM affects community assembly via niche filtering processes (i.e., by altering habitat suitability) because decreases in the convex hull volume imply less niche space available to be occupied (Cornwell et al. 2006, Mouchet et al. 2010).

We also detected increases in FDiv and FEve downstream of MTM (Table 3). Similar responses have been reported in association with increasing landuse intensity for plant (Pakeman 2011) and bird assemblages (Luck et al. 2013). Increasing FDiv values indicate that species characterized by extreme functional trait values (i.e., located near edge of convex hull in principal coordinates analysis) have become relatively more abundant (Villéger et al. 2008), and increases in FEve imply increasing functional separation

among species. Increases in these functional diversity indices have been interpreted as signals of competitive exclusion because abundances are highest where functional overlap among taxa is lowest (Mouchet et al. 2010). Alternatively, increases in FEve and FDiv may be a secondary consequence of reduced FRic values because this reduction limits the convex hull volume available for FEve and FDiv values.

Observed effects of MTM could not be explained by changes in physical habitat conditions (Fig. 3). Moreover, greater thalweg depths at SE than SR (Fig. 3A) would be expected to increase species richness, but the opposite pattern was observed. In contrast, differences in water quality between exposed and reference sites corresponded with observed patterns in fish assemblage. Effects of MTM on fish assemblages might be linked to effects of water quality on prey resources for fishes. The transition from reference to exposure fish assemblage composition occurred at conductivities between ~600 and 1000  $\mu\text{S}/\text{cm}$  (Fig. 2A). These values exceed conductivity levels expected to reduce benthic macroinvertebrate diversity in Appalachian streams (Pond et al. 2008, Pond 2010, Merriam et al. 2011). USEPA (2011a) identified a protective benchmark for benthic macroinvertebrate assemblages at 300  $\mu\text{S}/\text{cm}$ , and our results suggest that the thresholds for fishes will be somewhat higher.

Decreases in abundances of obligate invertivores at TE (Fig. 5B) suggest that changes in fish assemblages might be a consequence of decreased availability of invertebrate prey. This pattern was most evident for darters (Percidae), which exhibited a monotonic decrease in abundance from 214 individuals in 1999 to 5 individuals in 2011 (Appendix S2). Similar losses of darter species richness and abundance have been reported downstream of MTM operations in Twentymile Creek, West Virginia (F. Borsuk, USEPA, personal communication), and Hopkins and Roush (2013) reported decreasing occurrence probabilities for Frecklebelly Darter (*Percina stictogaster*) and Eastern Sand Darter (*Ammocrypta pellucida*) (but not Kentucky Arrow Darter [*Etheostoma spilotum*]) downstream of MTM operations in eastern Kentucky. Additional research is needed to assess how reduced diversity of macroinvertebrate assemblages affects their total biomass and how this, in turn, affects prey selection by stream fishes.

Prey quality also may be altered by Se exposure and assimilation dynamics. The transition from reference to exposure fish assemblage composition occurred at total dissolved Se concentrations between 4 and 7  $\mu\text{g}/\text{L}$ , a range that encompasses the EPA's national recommended water-quality criterion for aquatic life (5  $\mu\text{g}/\text{L}$ ). Lemly and Skorupa (2007) recommended that a total dissolved Se concentration = 2  $\mu\text{g}/\text{L}$  should be used as a threshold to trigger tissue-based analyses of Se exposure risks. However, we did not measure dietary Se, which is more directly linked to toxicological endpoints than dissolved concentrations (Stewart et al. 2010), so we cannot offer a complete perspec-

tive on Se hazards for stream fishes downstream of MTM. McDonald and Chapman (2007) recommended population trend analyses for site-specific assessments of Se contamination, and we cannot reject the possibility of Se-induced recruitment failures. However, intra- and interspecific variation in fish responses to Se and the lack of sufficient population-level data may limit the utility of population analyses in this regard. We recommend gut-content analysis of the small-bodied fishes preserved by Stauffer and Ferreri (2002) and in our current study to investigate the relative importance of prey quality (Se exposure) and prey availability (energetic limitations).

Our fish biomass data also suggest an influence of trophic mechanisms on fish assemblage structure. Observed increases in mean individual biomass (Fig. 4B) occurred contemporaneously with decreases in total fish abundance at TE (Table 3), indicating that decreased interspecific competition may facilitate per capita growth for tolerant species. Self-thinning models are commonly used to model the relationship between population density and per capita growth (Dunham and Vinyard 1997) and have been applied to estimate effects of MTM on sport fishes (McGarvey and Johnston 2013). Our results provide an assemblage-level analogue for self-thinning, but not a self-thinning dynamic per se because the species primarily responsible for increases in mean individual biomass (*L. cyanellus* and *S. atromaculatus*) did not decrease in abundance over time (Appendix S2). Nonetheless, persistence of *L. cyanellus* and *S. atromaculatus* may indicate energetic limitations because adults of these species include fish in their diet (Jenkins and Burkhead 1994), which might relax the requirement for macroinvertebrate prey that restricts other taxa in MTM-affected streams. The increasing dominance of piscivorous *L. cyanellus* and contemporaneous declines of other species also could signal predation effects, as has been observed elsewhere (Lemly 1985), but this explanation is unlikely by itself because *L. cyanellus* was present in the samples collected by Stauffer and Ferreri (2002).

Stream fish assemblages are expected to change over time as individuals move among stream network locations to track resource availability and escape locally unsuitable habitat conditions (Schlosser 1991). We observed temporal variation at TR that could have indicated recovery from prior drought conditions. The collections by Stauffer and Ferreri (2002) made in 2001 followed the most extreme drought conditions during 1999–2000 (Adams and Warren 2005, USEPA 2005), but fish assemblage recovery to predrought conditions might require months or years (Larimore et al. 1959, Matthews and Marsh-Matthews 2003, Adams and Warren 2005). TR was ~1 fkm upstream from the confluence of Laurel Creek and Big Ugly Creek (upstream basin area = 58  $\text{km}^2$ ) and 7 fkm upstream from the confluence with the Guyandotte River (upstream basin area ~2700  $\text{km}^2$ ; Fig. 1). Riverine connectivity within this distance is expected to increase fish richness and abun-

dance, particularly for cyprinid taxa (Gorman 1986, Hitt and Angermeier 2008a, 2011), and we observed several-fold increases in cyprinid abundance and biomass over time at TR (Appendix S2). *Cyprinella spiloptera* was not detected in 2001 but made up 44% of fish abundance at this site in 2011 (Appendix S2). In contrast, TE lacked such riverine connectivity because of the presence of the Mud River reservoir and the absence of large connected streams (Fig. 1). The lack of connectivity to high-quality source populations may help explain the lack of biological recovery downstream from MTM operations (Merovich et al. 2013).

Study design limitations should be considered when interpreting our results. We used electrofishing to sample stream fishes, and this technique loses efficiency in high-conductivity water (Hill and Willis 1994). However, we cannot attribute observed differences to sampling efficiency alone because the original surveys also were done in high-conductivity water (Table 1). Moreover, we did not observe herding of fish ahead of the electrofishing field and did not detect additional species in supplemental seining outside the sample reach. We recommend a rotenone-sampling experiment to quantify electrofishing efficiencies across the observed conductivity gradient. Functional diversity indices can be limited by the choice of species traits for analysis (Petchey and Gaston 2006), and another selection of species traits could yield different results. However, we expect our functional diversity results to be robust because selected traits included measures of body size, fecundity, and parental care that have been useful for partitioning total variation in fish life-history strategies across North America (Winemiller and Rose 1992). The geographic restriction of our study area to the Guyandotte River basin limited confounding physiographic and zoogeographic effects, but necessitates additional research to evaluate temporal trends elsewhere.

Our study design and analytical approach permitted new inferences regarding effects of MTM. Our choice of study sites provided results that were largely unconfounded by residential development, so we avoided a complication encountered in prior studies (Fulk et al. 2003; see also Merriam et al. 2011). The Left Fork Mud River site was particularly useful in this regard because it is a relatively large stream that lacks headwater mining in the study area (35 km<sup>2</sup> upstream basin area; Table 1). Fulk et al. (2003) limited their inferences regarding MTM to watersheds <10 km<sup>2</sup>, whereas each site in our analysis drained watersheds at least twice this size (Table 1). Our approach also benefited from the use of null assemblage simulations to identify effects of MTM that could not be attributed to random variation in fish assemblage composition. Similar community simulation approaches have been useful for analysis of abundance trends (Gotelli et al. 2010) and functional diversity (Mason et al. 2012). Our analysis suggested that

changes in fish assemblages may be more attributable to dietary and energetic mechanisms (prey quality and availability) than physical-habitat alterations downstream from MTM.

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