Characterizing coal and mineral mines as a regional source of stress to stream fish assemblages

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ABSTRACT

Mining impacts on stream systems have historically been studied over small spatial scales, yet investigations over large areas may be useful for characterizing mining as a regional source of stress to stream fishes. The associations between co-occurring stream fish assemblages and densities of various "classes" of mining occurring in the same catchments were tested using threshold analysis. Threshold analysis identifies the point at which fish assemblages change substantially from best available habitat conditions with increasing disturbance. As this occurred over large regions, species comprising fish assemblages were represented by various functional traits as well as other measures of interest to management (characterizing reproductive ecology and life history, habitat preferences, trophic ecology, assemblage diversity and evenness, tolerance to anthropogenic disturbance and state-recognized game species). We used two threshold detection methods: change-point analysis with indicator analysis and piecewise linear regression. We accepted only those thresholds that were highly statistically significant ($p \leq 0.01$) for both techniques and overlapped within $\leq 5\%$ error. We found consistent, wedge-shaped declines in multiple fish metrics with increasing levels of mining in catchments, suggesting mines are a regional source of disturbance. Threshold responses were consistent across the three ecoregions occurring at low mine densities. For 47.2% of the significant thresholds, a density of only ≤ 0.01 mines/km^2 caused a threshold response. In fact, at least 25% of streams in each of our three study ecoregions have mine densities in their catchments with the potential to affect fish assemblages. Compared to other anthropogenic impacts assessed over large areas (agriculture, impervious surface or urban land use), mining had a more pronounced and consistent impact on fish assemblages.

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1. Introduction

Studies describing responses of stream biota to coal and mineral mines located in stream catchments have historically been conducted at the scale of a single stream or river basin. For example, Schorr and Backer (2006) studied effects of coal mine drainage on fish assemblages in a single stream in Tennessee, while Freund and Petty (2007) characterized how mining-related pollutants were dominant factors leading to degradation of stream fish and macroinvertebrate assemblages in a single basin in West Virginia. Despite the fact that their work was conducted within a small area, Freund and Petty (2007) suggested that mining may have wide-spread, regional influences on stream systems, including affecting streams lacking mines in their catchments but having hydrologic connections to streams with mined catchments. Such regional influences may include restricted passage for organisms throughout river networks as well as reduced regional species pools resulting from extreme modifications to stream habitats. Few studies have specifically examined disturbances resulting from mining over multiple basins (but see Maret et al., 2003; Wickham et al., 2007; Townsend et al., 2009), and few have specifically considered responses of stream fishes at large spatial

scales (Hughes, 1985). While site-specific knowledge is valuable for characterizing mechanistic effects of mines on streams, regional-scale studies may aid in characterizing cumulative impacts to ecosystems (Karelva and Wennergren, 1995) and reveal regional stresses to stream fish assemblages.

Various types of mining activities, including mineral and coal mining as well as supporting activities such as drill holes or cores, can have similar effects on streams as other anthropogenic land uses like urbanization and agriculture. Mining can alter catchment hydrology because mine development represents an alteration from natural land covers (Bernhardt and Palmer, 2011; US EPA, 2011). Mined catchments have been shown to respond to precipitation in a manner similar to urbanized catchments, including having flashy stream flows resulting from altered landscapes (Phillips, 2004; Bernhardt and Palmer, 2011; US EPA, 2011). Hydrologic flashiness of streams in catchments can change stream habitats (Brim Box and Mossa, 1999; Bernhardt and Palmer, 2011) and disrupt fish life cycles and cohort recruitment (Kohler and Hubert, 1999). Mined catchments can have altered channel morphology as a result of increased sedimentation and altered alluvial deposition patterns (Brown et al., 1998; Brim Box and Mossa, 1999), loss of riparian buffers (Bernhardt and Palmer, 2011) and/or channel loss from mountain top removal mining operations that deposit overburden rock and soil into adjacent valleys (Bernhardt and Palmer, 2011; US EPA, 2011). Mining activities can alter food for fishes by hindering detrital processing (Word, 2007; Fritz et al., 2010) or by shifting food webs from detrital-based to primary production as forested headwaters are lost (Hill et al., 1995; US EPA, 2011). Mining may degrade macroinvertebrate (Hartman et al., 2005; Pond et al., 2008) and algal assemblages (Wissmar, 1972), which may ultimately limit the biomass of fishes and other organisms in stream systems (US EPA, 2011).

Mining, however, may also alter stream systems via a unique set of influences that differ from other anthropogenic land uses. Mining can expose un-weathered materials to the atmosphere. These materials may become a source of water pollution via mine drainage runoff, characterized by high concentrations of metals, high conductivity, excess sediment and in sulfide-rich spoils, low pH levels (Hartman et al., 2005; Schorr and Backer, 2006; Pond et al., 2008; US EPA, 2011). Reduced fish and macroinvertebrate survival and production have been found in streams receiving mine drainage (Letterman and Mitsch, 1978; Howells et al., 1983). The accumulation of both influences from anthropogenic land use plus the unique effects from mines reduces fish species richness and abundance (Letterman and Mitsch, 1978; Howells et al., 1983; Ferreri et al., 2004; Schorr and Backer, 2006; US EPA, 2011).

Considering relationships between stream fishes and mine densities within a range of stream sizes may be an effective strategy for understanding cumulative disturbance of mines across large regions. Fish are differentially sensitive to various anthropogenic disturbances (Karr et al., 1986), and some species may disperse from disturbed habitats to access favorable locations to survive or complete their life cycles (Kohler and Hubert, 1999). Fish assemblages in disturbed habitats may have reduced numbers of species or individuals compared to assemblages in unaffected habitats. When examined over large regions, differences in assemblages can indicate differences in stream habitats resulting from disturbances, including mines (Karr et al., 1986; Kohler and Hubert, 1999; Flotemersch et al., 2006). Fish are also relatively easy to collect and identify in the field, and they have a high social and cultural value (Flotemersch et al., 2006). Due to differences in regional species pools, however, assessing influences on fish assemblages resulting from disturbances over large regions may be best accomplished by considering associations of assemblages summarized by functional traits such as feeding strategies, habitat preferences, or stressor tolerance of species vs. species-specific

metrics (Poff and Allan, 1995). For instance, loss of lithophilic spawners suggests that fish assemblages may be influenced by hydrologic alteration (Grabowski and Isley, 2007) and/or siltation of coarse substrates (Berkman and Rabeni, 1987). Consideration of changes in functional traits of fishes with disturbances is the basis for monitoring biological integrity of stream systems (Karr et al., 1986; Karr, 1991) and for stream assessment efforts (e.g., Esselman et al., 2013), as it yields insights into where and how to prioritize management actions for conservation of species of interest.

The goal of this study was to characterize associations between mines and stream fish assemblages in numerous streams in three large ecoregions, to determine whether mines are a regional source of stress. We address three objectives in our study. First, we tested for associations between stream fish assemblages and densities of various "classes" of mining occurring in their catchments. As this occurred over large regions, we summarized fish assemblages by various functional traits as well as other measures of interest to management. Second, we compared responses of specific fish metrics to mine densities across regions to evaluate consistency in detected associations. Finally, to lend support to understanding of mines as a regional source of stress to fish, we compared responses of three selected fish metrics found to respond negatively to mining to urban, agricultural and impervious land covers within the same regions.

2. Methods

2.1. Study regions and spatial framework

We conducted our study within three ecoregions in the eastern portion of the United States, selected from aggregated ecoregions used in the USEPA's National Wadeable Streams Assessment (WSA) (US EPA, 2006) and the 2010 National Fish Habitat Partnership (NFHP) inland assessment (Esselman et al., 2013). The Northern Appalachian ecoregion (NAP), Southern Appalachian ecoregion (SAP) and Temperate Plains ecoregion (TPL) (Fig. 1) were selected because they had at least 1000 stream reaches with both sampled fish assemblages and a range of density of mines in stream catchments. The area of regions, percentage of various anthropogenic land use/land covers within each region and the number of mines in each region are presented in Table 1.

The base data layer used for this study was the National Hydrography Dataset Plus Version 1 (NHDPlusV1) (NHDPlus, 2008), which includes 1:100,000-scale river arcs, referred to as stream reaches, as the smallest spatial unit for summarizing and analyzing data in this study. We used stream reaches from the NHDPlusV1 that extend from confluence to confluence or to junctions with lakes or reservoirs. In headwater streams, the origin of streams serves as the beginning of reaches that end at their first confluence or junction with a lake or reservoir. This network of confluence to confluence reaches extends to the mouths of rivers for the entire study region (Esselman et al., 2011; Wang et al., 2011). Landscape data, including mine densities, were summarized at two spatial scales. "Local catchments" include all land area that drains directly into an individual stream reach and "network catchments" include upstream lands throughout the stream network, including the local catchment (Wang et al., 2011).

The percentages of urban and agricultural land uses and impervious surfaces were summarized within local and network catchments from the 2001 National Land Cover Dataset (2001 NLCD) (Homer et al., 2007). Urban land use includes the sum of percentages of open space, low, medium and high density urban lands, and agricultural land use includes the sum of percentages of pasture and row crops. Six natural landscape variables were also summarized for each ecoregion using geographic information system software (ESRI, 2011): network
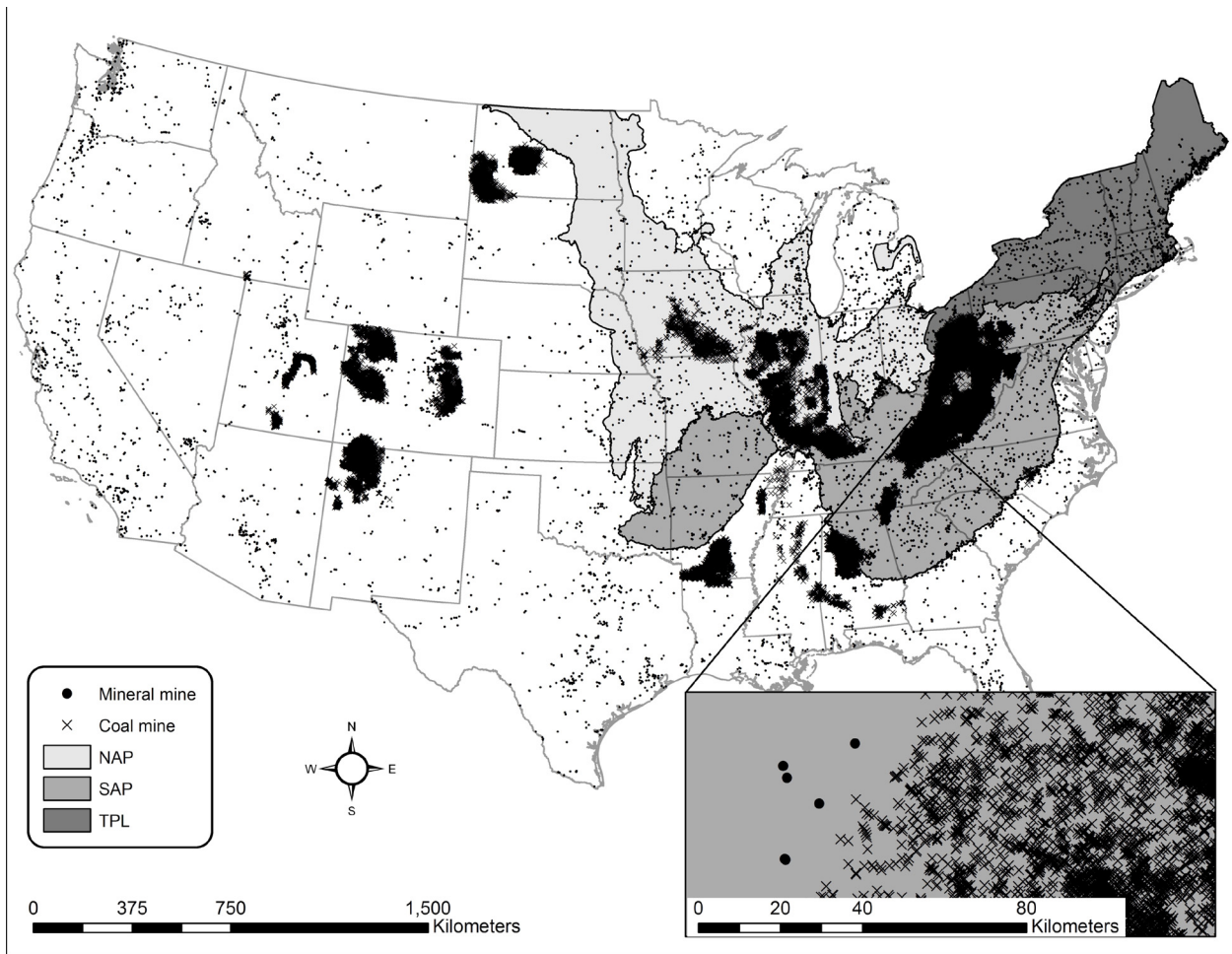
Fig. 1. Coal and mineral mines in the conterminous United States. All points correspond to mine location. Black points represent mineral mines and processing plants and black Xs represent major coal mines and minor coal mine activities. Inset map shows separation of mine points over smaller scaled area. Outlined ecoregions correspond to US EPA's National Wadeable Streams Assessment (US EPA, 2006) ecoregions: NAP = Northern Appalachian Ecoregion, SAP = Southern Appalachian Ecoregion and TPL = Temperate Plains Ecoregion.

catchment area and five local catchment variables including mean catchment slope (degrees), mean annual precipitation (mm), mean catchment elevation (m), groundwater index (% groundwater contribution to baseflow) and soil permeability rate (inches/hours × 100). Mean catchment slope and elevation were acquired from the national elevation dataset (USGS, 2005). Mean annual precipitation and catchment area were from the NHDPlusV1. Groundwater index was from a base-flow index grid developed for the conterminous United States from USGS (Wollock, 2003), and soil permeability rates were calculated from State Soil Geographic (STATSGO) Data Base (USDA, 1995).

2.2. Mine data

Locations and number of mineral and coal mines were compiled from two sources (Fig. 1). Locations of mineral mines and processing plants were from the USGS Mineral Resources Program (USGS, 2003), a database consisting of point data locations of non-energy mining actives including gravel and precious and non-precious mineral mining and processing. Coal mine data were provided from the Survey National Coal Resources Data System Stratigraphic (USTRAT) database (USGS, 2012). The USTRAT incorporates locations of coal mine sites and mining support activities occurring in most states since 1975. Besides locational

Table 1
Characteristics of the Northern Appalachian (NAP), Southern Appalachian (SAP) and Temperate Plains (TPL) ecoregions. Ecoregion area is from US EPA's National Wadeable Streams Assessment (US EPA, 2006), and total stream length within each ecoregion was determined from the NHDPlusV1 stream layer. Total number of mineral mines is from USGS Mineral Resources Program (USGS, 2003), and total number of coal mines (minor and major) is from USTRAT database (USGS, 2012). Highest mine density was calculated for network catchments. Average percentages of agricultural and urban land use and impervious surface within each region were derived from the 2001 NLCD.

WSA region	Region area (km^2)	Stream length (km)	Mineral mines (#)	Major coal mines (#)	Minor coal mines (#)	Highest mine density (mines/km^2)	% agriculture land use	% impervious surface	% urban land use
NAP	355,944	252,990	714	1,041	180	39.4	14.84	2.54	10.13
SAP	836,025	707,996	1,374	55,670	35,778	8.5	22.21	1.53	8.20
TPL	888,794	649,719	1,190	1,373	39,285	3.8	61.13	1.60	7.81

information, USTRAT data included two classes of mine types, major mining activities (e.g., surface mining, prospect pit, underground mine, etc.) or minor support activities for mining (e.g., drill hole, core, water well, etc.). Those two USGS defined classes of coal mines were retained for this study (USGS, 2012). Mine types were summarized into five classes for analysis: mineral mines and processing plants, major coal mines, minor coal mine support activities (referred to as minor coal mines), all coal mines (combined major and minor mines) and total mines (combination of mineral and coal mines). All mine classes were summarized as a density (#/km^2) in both local and network catchments to test for potential differences in effects of mines on reaches supporting stream fishes.

2.3. Fish indicators of habitat quality

Data characterizing stream fish assemblages were compiled from state and federal programs and spatially referenced to corresponding stream reaches of the NHDPlusV1. Fish data per reach included abundance measurements of fishes identified to species from assemblage sampling (versus sampling that targeted specific species) from 1990 to 2010. All data were collected using single pass electrofishing. We used a single sample from a single year to represent a reach.

Fish assemblages were summarized into 10 metrics that were reported in the literature as responsive to disturbance resulting from mining (Letterman and Mitsch, 1978; Maret and MacCoy, 2002; US EPA, 2011) and representing important management considerations. Ideal fish assemblage metrics for characterize associations with mines will capture information about the complex structural and/or functional alteration from anthropogenic disturbance, be predictable in their response and provide insights for management actions (Dale and Beyeler, 2001). Metrics were grouped into 6 categories based on their representation of various ecological factors and management considerations. Factors include (1) reproductive ecology and life history, (2) habitat preferences, (3) trophic ecology, (4) assemblage diversity and evenness, (5) tolerance to anthropogenic disturbance and (6) state-recognized game species with justification for consideration of metrics in each category to follow.

2.3.1. Reproductive ecology

Percent lithophilic spawning individuals (abbreviation Lith) as defined by Frimpong and Angermeier (2009) are species of fishes that spawn on or in clean gravel or cobble. Numerous species of darters (Percidae: Etheostomatini), minnows (Cyprinidae), suckers (Catostomidae) and salmonids (Salmonidae) are lithophilic spawners. Fishes having this reproductive strategy may be vulnerable to water fluctuations (Grabowski and Isley, 2007) and to siltation of coarse substrates (Berkman and Rabeni, 1987). Mining may cause both hydrologic modifications (Bernhardt and Palmer, 2011; US EPA, 2011) and increases in siltation (Brown et al., 1998; Brim Box and Mossa, 1999) in stream systems, and the influence of mining may be reflected in decreased abundances of lithophilic spawners in streams with mined catchments.

2.3.2. Habitat preferences

Percent native rheophilic taxa (abbreviation Rheo) as defined by Frimpong and Angermeier (2009) are species that prefer fast-flowing water habitats. This metric includes a variety of species preferring riffles and includes salmonids. This metric is of importance because mining can alter stream hydrology by either increasing peak flows beyond natural conditions (Bernhardt and Palmer, 2011), or decrease flows as the water table is lowered (Younger and Wolkersdorfer, 2004). Reduced abundances of rheophilic taxa in streams draining catchments with mines may indicate a response to altered flows from mining.

2.3.3. Trophic ecology

Three trophic metrics (percent herbivore individuals, percent invertivore individuals and percent native piscivore taxa) as defined by Frimpong and Angermeier (2009) were selected to test for diversity of feeding groups to mines in catchments. The piscivore metric (abbreviation Pisc) measures the abundance of top carnivores in reaches, with higher values indicating higher trophic level stability. The invertivore metric (abbreviation Invert) measures the lower trophic levels. Because invertebrates are sensitive to mining activities (Hughes, 1985; Hartman et al., 2005; Pond et al., 2008), decreases in this metric with mines may suggest an effect from habitat degradation or food availability, potentially resulting from mining. Percent herbivore individuals (abbreviation Herb) are primary consumers, and changes in their abundances can potentially demonstrate shifts from allochthonous-based to autochthonous-based food webs from the loss of woody riparian zones.

2.3.4. Assemblage diversity and evenness

Assemblage responses to mining also were measured with Shannon's diversity index (abbreviation H′; H′ = −SUM{pi × ln(pi)}; Shannon and Weaver, 1963) and assemblage evenness (abbreviation J′; J′ = H′/ln S; Pielou, 1977). Both metrics were calculated for each fish sample based on Hauer and Lamberti (2007). Reductions in these values with increasing levels of mining may suggest impairments in fish assemblage structure, including alteration away from best available conditions (Mol and Ouboter, 2004).

2.3.5. Tolerance to anthropogenic disturbance

Reduced abundances of intolerant individuals with increasing levels of anthropogenic disturbance in stream catchments may be an indicator of impaired habitat quality, while increased percent tolerant individuals with increasing levels of disturbance may indicate an abundance of common, hardy species that can survive in degraded habitats. Intolerant (abbreviation Intol) and tolerant species metrics (abbreviation Tol) were developed from the US EPA's published list of fish indicator species identified by seven independent biological integrity assessments across the eastern US (Grabarkiewicz and Davis, 2008). Species included in this national intolerance list were incorporated into this metric based on each separate assessment's designation for a given species along a gradient of tolerance. We chose the most conservative species from the list that had the majority of their designations as either tolerant or intolerant, but not both (Appendix A and B).

2.3.6. Game species

Game fishes (abbreviation Game) are defined in this study as species (or in some cases, groups of fishes) that are recognized by individual states as potentially being targeted by anglers and that have regulations limiting their harvest for recreational use as described in publically-available fishing guide books specific to each state. The purpose for generating this metric is to test responsiveness of game fishes to mining, as this may be of special interest to state managers within study regions. This metric was applied specifically to each state and reflects only that state's recognized game species.

2.4. Spatial analysis

Spatial autocorrelation occurs when sample sites cluster over small spatial or temporal scales, causing a lack of independence (Schabenberger and Gotway, 2005). To check for spatial autocorrelation and type I error, we conducted spatial analysis of our fish

assemblage sampling locations in each of the three ecoregions using Spatial Analysis in Macroecology program (SAM, Version 4.0; Rangel et al., 2010). The SAM model of simultaneous autoregression (SAR) uses GPS coordinate information or geographical distance values of variables retained to assess the independence of the sites (Dormann et al., 2007). To determine the amount of spatial autocorrelation within an ecoregion, we used the latitude and longitude of sampling locations, network catchment area, at the scale of local catchment mean catchment slope, mean annual precipitation, mean catchment elevation, groundwater index and soil permeability for each reach with a fish sample. Use of latitude and longitude is a conservative approach for testing for spatial autocorrelation, as true network distance could result in longer pair-wise distances between sample points and potentially less spatial autocorrelation among points. The developed Moran-I values from the procedure were graphed in correlograms and inspected for distance of spatial independences. For Moran I values that indicated positive autocorrelation (Schabenberger and Gotway, 2005), a set of eigenvector-based spatial filters were applied within the SAM procedure. Eigenvector filters were selected to lower Akaike information criterion (AIC) values, to remove as much of the autocorrelation as possible. We retained the residuals from the SAM procedure for further analyses.

2.5. Variation in mines across catchment sizes

Fish assemblages in catchments with smaller drainage areas may be more strongly influenced by the surrounding landscape than assemblages in catchments with larger drainage areas (Vannote et al., 1980; Allan, 2004). We evaluated the total mines density across different stream size strata and catchment position within the network catchment for each ecoregion. Six catchment size categories were used to group all catchments based on Goldstein and Meador (2004) and Wang et al. (2011) and include: headwaters $<10\,km^2$, creeks $10<100\,km^2$, small rivers $100<1,000\,km^2$, medium rivers $1000<10,000\,km^2$, large rivers $10,000<25,000\,km^2$ and great rivers $25,000\,km^2$.

2.6. Testing for associations between mines and fish

To account for known influences of catchment area, slope, elevation, precipitation and groundwater inputs to streams on distributions and abundances of stream fish assemblages, we controlled for these natural factors in analysis following Esselman et al. (2013). We developed boosted regression tree models (Elith et al., 2008) for each assemblage metric in each ecoregion using least-disturbed sites from the ecoregion and important natural landscape predictors. Boosted regression tree models were used to predict the 10 metric scores that would be expected under least-disturbed conditions at all sites. A least-disturbed site had to meet five criteria: land use in the catchment had to be $<1\%$ urban land use, $<10\%$ catchment pasture, $<10\%$ row crop, $<0.5\%$ impervious surface and the assemblage metric could not have a zero value. We used the same six variables used in the spatial analysis for modeling natural gradients in each ecoregion (network catchment area, at the scale of local catchment mean catchment slope, mean annual precipitation, mean catchment elevation, groundwater index and soil permeability). We tailored the learning rate and tree complexity of each model to minimize prediction error after a minimum of 2000 iterations. The initial tree complexity was set at 5 with a bag ratio of 0.5. The residuals from the boosted regression were rescaled from 0 to 100, except for H′ and J′ which were rescaled to their appropriate distribution and used in further statistical analyses.

Identification of ecological thresholds were conducted using a combination of two threshold analyses, change-point analysis

with indicator analysis and a piecewise linear regression which were used to generate more robust and precise composite prediction of the threshold response (Qian and Cuffney, 2011). Change-point analysis with indicator analysis was used to conduct a permutation test to determine the location of greatest difference of fish metric response to mine density. This nonparametric test identifies step thresholds. We used the R code TITAN (Baker and King, 2010) to determine the change-point threshold for the fish metrics for each mine class. Only the individual metrics' thresholds were used for these assessments instead of TITAN's community threshold. Change-point thresholds were recognized with a p-value ≤ 0.01. Piecewise linear regression (i.e., segmented regression) was used to partition fish metric values into two intervals that fit separate line segments and to identify the change-point or threshold boundary between the intervals. This parametric test recognizes hockey-stick or broken-stick thresholds (MacArthur, 1957). We used the R code package Segmented (Muggeo, 2013) to identify the breakpoint thresholds. Piecewise thresholds were recognized with p-value ≤ 0.01. To be considered a significant threshold, both the change-point and piecewise regression thresholds had to overlap within the $\leq 5\%$ error rate range. The combination of the nonparametric and linear threshold analysis along with the strict criteria of significance provided conservative estimates of fish metric-mine thresholds.

2.7. Testing for associations between mines and other disturbances

We compared the threshold responses of a subset of fish metrics to mine density to thresholds from other anthropogenic land use in catchments including urban land, agriculture and impervious surfaces. The same fish metric was plotted against each disturbance type; intolerant individuals were evaluated in the NAP, tolerant individuals in the SAP and game individuals in the TPL. Piecewise linear regression was applied to urban, agriculture and impervious surface with the same fish metrics. The thresholds were identified at a p-value of 0.05. To facilitate comparison, we transformed independent variables into z scores and projected them from 0 to 1.
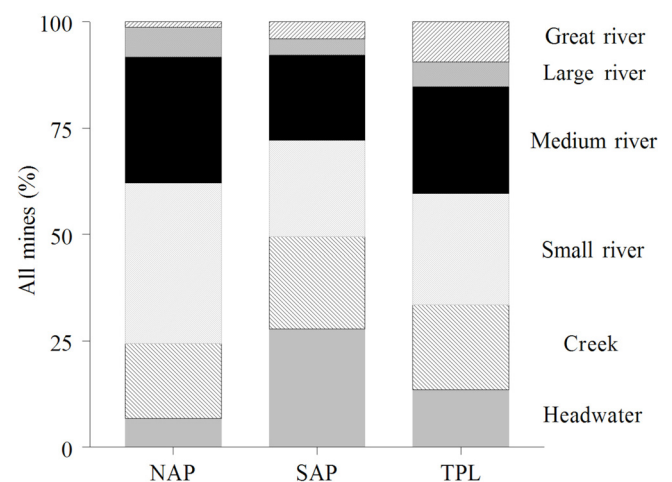
Fig. 2. Total mines class locations within stream sizes for the three ecoregions. Headwater $<10\,km^2$, creek $10<100\,km^2$, small river $100<1,000\,km^2$, medium river $1000<10,000\,km^2$, large river $10,000<25,000\,km^2$ and great rivers $25,000\,km^2$ (Goldstein and Meador, 2004; Wang et al., 2011).

3. Results

Mineral and coal mines were located in all three ecoregions (Table 1), but densities and counts of mines varied widely across catchment size classes. The NAP ecoregion had the smallest total number of mines (1935) but had the highest mine density in a network catchment (39.4 mines/km^2). The SAP ecoregion had the greatest number of total mines (92,822). The highest mine density in the SAP ecoregion was 8.5 mines/km^2 (network catchment). The TPL ecoregion had a total of 41,848 mines, with the highest mine density in a TPL network catchment of 3.8 mines/km^2. When considering size of the catchments that all of our mines and support activities occurred in (Fig. 2), the SAP ecoregion had a largest number of mines that occur in the headwaters (27.75%) and in creeks (21.72%). The combination of the small and medium river classes had the highest number of mines in the NAP ecoregion (67.4% combined). The large rivers and great rivers had the highest number of mines (15.19% combined) in the TPL ecoregion.

3.1. Threshold detection

Using our conservative criteria, 89 of the 300 tested relationships were significant, and with over half of significant thresholds (58.4%) occurring at densities $\leq$0.05 mines/km^2. These low density values suggest that a single mine can led to threshold responses. Also, each of the 10 fish metrics showed at least one threshold response with each mine class in at least one of the ecoregions (Table 2), the tolerant species metric had the most significant thresholds (15), and Shannon's diversity index had the most consistent response to mine densities with 53.8% of the significant thresholds occurring at a density less than 0.002 mines/km^2 in all regions. There were fish metric threshold responses to each class of mining, the number of threshold responses include: mineral mines (14), major coal mines (14), all coal mines (19), total mines (20) and minor coal mines (22) (Table 2).

Except for percent herbivore individuals in SAP, all metrics showed wedge-shaped declines in response to increasing mine density (plots not presented). Mine disturbances tested over both local and network catchments all revealed significant thresholds, but more occurred at the network than at the local catchment scale (66 vs. 23 cases). Most fish metrics that had significant threshold responses to mine density at the local scale (with 4 exceptions in the 23 cases) also had significant responses to the same mine class at the network scale within an ecoregion.

3.2. Regional response

3.2.1. NAP ecoregion

Among the 28 significant threshold responses identified, the highest threshold response occurred at 0.65 mines/km^2 for percent tolerant individuals for local major coal mines (Table 2). Of the significant threshold responses at the local and network scales, 55.2% occurred at densities <0.01 mines/km^2. In this ecoregion, local minor coal mine density did not have a threshold association with any of the fish metrics, and fish evenness (J') showed no association with any of the mine classes. At a local scale, piscivore, Shannon's (H') and game fish metrics had significant threshold responses to multiple mine classes including the mineral mines class (Fig. 3A). At the network scale, all but the evenness (J') metrics had significant thresholds with mine class densities. The most responsive fish metrics, defined by having the most threshold associations with the mine classes at both spatial scales, were the piscivore, Shannon's (H') and game fish metrics. The most common mine disturbance class for the ecoregion, defined by having the highest number of statistically significant fish metrics-mine

density threshold associations, was the total mines class at the network catchment scale.

3.2.2. SAP ecoregion

In SAP, the highest threshold was 1.20 mines/km^2 for percent intolerant individuals and network major coal mines (Fig. 3B). In this ecoregion, 57% of the significant threshold responses occurred at $\leq$0.05 mines/km^2 (Table 2). All fish metrics had a significant threshold response and only local mineral mine density failed to yield a significant threshold association with any fish metric. Herbivores in this ecoregion showed a positive relationship at the lower end of the gradient with network minor coal (Fig. 3C) and mineral mining. At the network catchment scale, all fish metrics responded to mine density with significant thresholds, but at the local catchment scale, the only significant threshold response was for the tolerant individuals metric. The most responsive metric for the SAP ecoregion was the tolerant fish metric. The most common mine disturbance classes were the network mineral mines and minor coal mines. SAP had the fewest significant threshold responses (23%) in the study, although this ecoregion contained the most mines.

3.2.3. TPL ecoregion

Fish metrics in the TPL ecoregion had the most threshold responses to mine classes at both local and network scales. A total of 38% of the threshold responses were significant, and 53% of those thresholds occurred at densities <0.05 mines/km^2 (Table 2). Percent intolerant individuals had the highest threshold response to mining at 0.175 local minor coal mines/km^2. There were no threshold associations between the fish metrics and local mineral and local major coal mine densities, and percent herbivores showed no significant threshold response to any type of mine density in the ecoregion. Invertivores showed significant threshold responses (Fig. 3D) for all tested mine classes except for local mineral and local, major coal mine densities. At the local scale, the most responsive metrics were lithophilic individuals, invertivore individuals, Shannon's (H') metric, intolerant individuals and tolerant individuals. All fish metrics, except herbivore individuals, had statistically significant threshold responses to mine classes/metrics summarized at the network scale.

3.3. Comparison to other disturbances

For the NAP, we compared intolerant individuals' responses to four disturbance gradients, transformed as z scores: density of total mines, percent urban land use, percent agriculture land use and percent impervious surface within network catchments (Fig. 4). The threshold location of intolerant individuals to the total mines class occurred at 0.001 mines/km^2. The urban and agricultural thresholds were higher (16.68 and 55.91% of land use, respectively). Impervious surface cover for this ecoregion did not produce a statistically significant threshold with any fish metric.

In the SAP, the most responsive fish metric was percent tolerant individuals (Fig. 4). The threshold response for the tolerant metric to the total mines class was at 0.42 mines/km^2, which was higher (when evaluated as z scores) than the threshold points for impervious surface at 1.45% of land cover and urban at 4.55% land use. The agricultural threshold was 9.3% land use, and when compared to other ecoregions, agricultural land use was the lowest.

Game individuals in the TPL ecoregion had a total mines threshold response of 0.001 mines/km^2; the lowest threshold value measured (Fig. 4). The ecoregion's impervious surface had a threshold of at 0.14% land cover, agricultural of 46.5% land use and urban at 15.66% land use.

Table 2

Significant threshold values for Northern Appalachian ecoregion (NAP), Southern Appalachian ecoregion (SAP) and Temperate Plains ecoregion (TPL). Threshold values shown (#/km^2) are from the piecewise linear regression procedure. Reference methods Section 2.3 for abbreviations. Metrics with the same threshold value are grouped within the disturbance class.

Disturbance classes	Ecoregion	Metrics	Thresholds	Relationship	Metrics	Thresholds	Relationship
			Local			Network	
Mineral mines	NAP	Pisc	0.0195	Negative	H′	0.68	Negative
					Game	0.001	Negative
	SAP	None	None	None	Lith, Rheo, Game	0.001	Negative
					Herb,H′	0.002	Positive, Negative
					Invert	0.001	Negative
					Pisc	0.007	Negative
	TPL	None	None	None	Lith, Game	0.001	Negative
					Invert	0.296	Negative
					H′	0.052	Negative
Minor Coal	NAP	None	None	None	Lith	0.009	Negative
					Herb	0.001	Negative
					Game	0.112	Negative
	SAP	Tol	0.246	Negative	Lith	0.003	Negative
					Rheo	0.021	Negative
					Invert	0.019	Negative
					Herb	0.021	Positive
					Pisc	0.012	Negative
					H′	0.004	Negative
					Tol	0.067	Negative
	TPL	Lith	0.121	Negative	Lith	0.1	Negative
		Invert	0.04	Negative	Invert	0.079	Negative
		H′	0.173	Negative	H′, J′	0.002	Negative
		Intol	0.089	Negative	Intol	0.001	Negative
		Tol	0.574	Negative	Tol	0.009	Negative
Major Coal	NAP	Pisc,	0.315	Negative	Lith	0.023	Negative
		Game	0.051	Negative	Herb, H′	0.001	Negative
		Tol	0.777	Negative	Game	0.14	Negative
	SAP	Tol	0.098	Negative	J′	0.647	Negative
					Intol	1.198	Negative
					Tol	0.101	Negative
	TPL	None	None	None	Rheo	0.134	Negative
					Invert, Intol	0.001	Negative
All Coal	NAP	Game	0.065	Negative	Lith	0.027	Negative
					Herb, H′	0.002	Negative
					Game	0.003	Negative
	SAP	Tol	0.091	Negative	Tol	0.141	Negative
	TPL	Lith	0.192	Negative	Lith	0.231	Negative
		Invert	0.049	Negative	Invert, Tol	0.001	Negative
		H′	0.096	Negative	Intol	0.08	Negative
		Intol	0.091	Negative	Pisc	0.009	Negative
		Tol	0.114	Negative	H′ J′	0.002	Negative
Total Mines	NAP	Pisc	0.02	Negative	Rheo, Pisc	0.005	Negative
		H′	0.001	Negative	Herb, H′, Tol	0.002	Negative
		Game	0.178	Negative	Invert	0.003	Negative
					Intol	0.001	Negative
	SAP	Tol	0.233	Negative	Tol	0.161	Negative
	TPL	Invert	0.09	Negative	Lith	0.229	Negative
					Invert	0.03	Negative
					Game, H′, Tol	0.001	Negative
					J′	0.029	Negative
					Intol	0.138	Negative

4. Discussion

Our study is one of the first to consider fish responses to both coal and mineral mine disturbances, independently and combined, in many streams over multiple large regions. Our results suggest mining of multiple types leads to a regional source of disturbance to fish assemblages. Almost every fish metric had a negative wedge response to increasing densities of mines and a threshold response to mines in at least one of the three ecoregions tested. This demonstrates that mines can influence stream fish assemblages in multiple ways, including affecting assemblage diversity and evenness; numbers of game species; and numbers of species with varied habitat preferences, trophic and reproductive strategies, and tolerance to stressors (Karr et al., 1986; Marzin et al., 2012).

Furthermore, for just under half of the significant metric thresholds detected (47.2%), a threshold response was found to occur at a density of <0.01 mines/km^2, suggesting that the inclusion of very low numbers of mines in stream catchments can negatively alter fish assemblages.

The literature has clearly established how mining can negatively affect stream fish assemblages, but many of these studies were conducted at smaller spatial scales and tested relatively localized effects of mining. The large number of statistically significant thresholds, we found at the network catchment scale indicates that downstream fish assemblages may be influenced by upstream mining, suggesting that mining can be a regional source of stress to stream fishes. Roughly 25% of NAP streams, 34% of TPL streams and 50% of SAP streams have mine
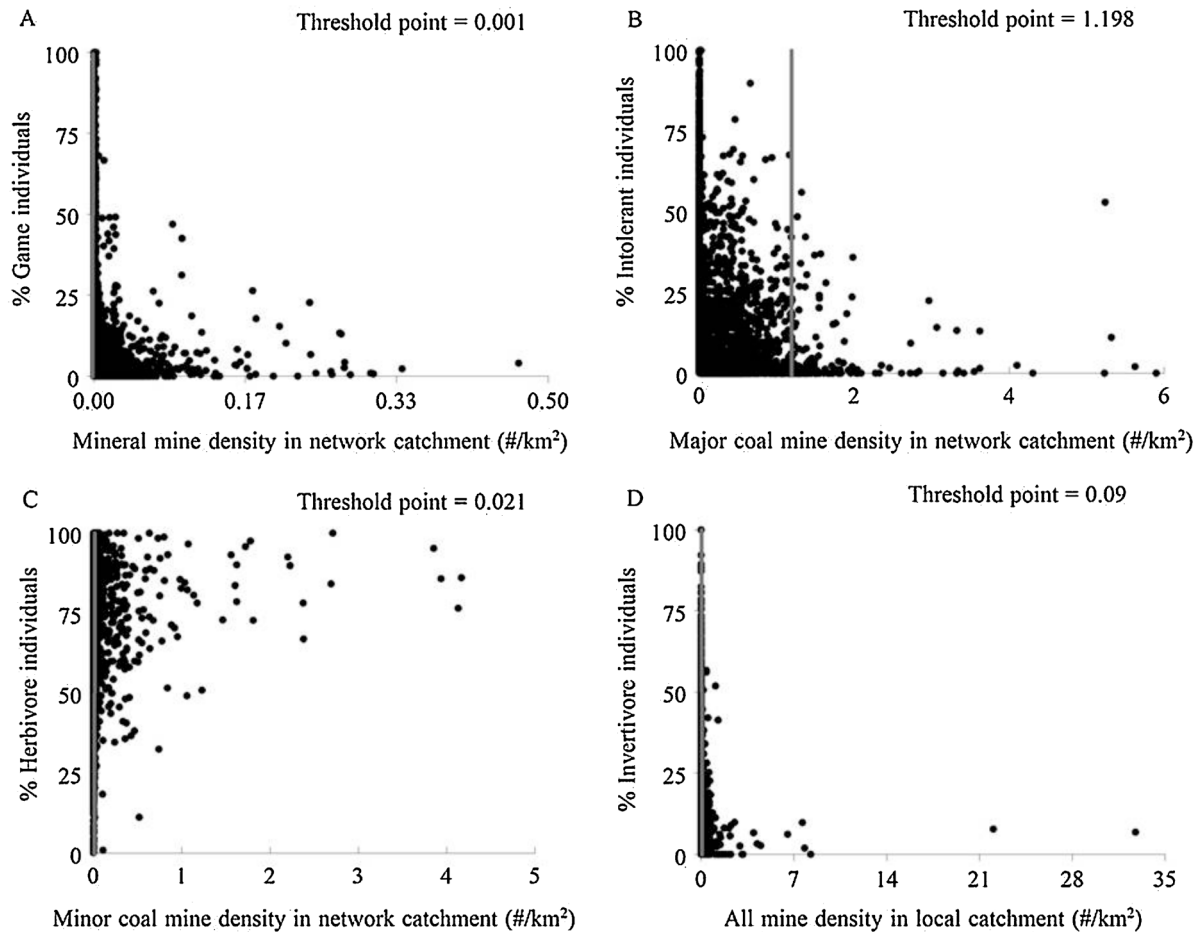
Fig. 3. Examples of thresholds and wedge-shaped responses to mine categories in the three ecoregions. Vertical charcoal line shows location of the threshold. (A) Percent game individuals associated with network mineral mines in the NAP ecoregion ($n = 7816$). Threshold occurs at the 0.001 mines/km². (B) Percent intolerant individuals associated with network major coal mines in the SAP ecoregion ($n = 7619$). Threshold occurs at the 1.198 mines/km². (C) Percent herbivore individuals associated with network minor coal mines in the SAP ecoregion ($n = 7619$). Threshold occurs at the 0.021 mines/km², wedge shape is positively correlated. (D) Percent invertivore individuals associated with local total mines in the TPL ecoregion ($n = 6355$). Threshold occurs at the 0.09 mines/km².

densities in their catchments with the potential to affect fish assemblages.

4.1. Ecological indicators of mining

Fish metrics that are consistently sensitive to disturbance can provide managers with information about alteration of habitat condition from mining (Dale and Beyeler, 2001). The most sensitive groups of fishes to mining, based on threshold point and repeatability in trends across regions, can be considered ecological indicators of mining. Invertivores, lithophilic fishes, tolerant fishes, intolerant fishes, fish diversity (H′), evenness (J′) and game species showed high sensitivity to increased mine density. This set of metrics can provide information about water quality (Grabarkiewicz and Davis, 2008), habitat and food web alteration (Hughes, 1985; Hartman et al., 2005; Pond et al., 2008), community structure (Mol and Ouboter, 2004), impacts from sedimentation (Brown et al., 1998; Brim Box and Mossa, 1999; Mol and Ouboter, 2004).

Many studies have found that tolerant species generally increase in disturbed systems (Boet et al., 1999; Onorato et al., 2000), but we found the opposite trend when considering mine density. For example, previous studies within single basins showed that mine drainage caused higher abundances of Centrarchids like green sunfish (*Lepomis cyanellus*) and bluegill (*Lepomis macrochirus*) (Jenkins and Burkhead, 1993; Schorr and Backer, 2006) as

well as creek chub (*Semotilus atromaculatus*) (Letterman and Mitsch, 1978; Schorr and Backer, 2006). These species have been reported as tolerant to mine drainage and associated pH shifts in streams (Jenkins and Burkhead, 1993). More tolerant species may occur at low levels of mine disturbance, but our tolerance metric, comprised of the above species and others (Appendix A), indicated a negative response to mine density, suggesting that mining impacts may accumulate downstream and have more pronounced influences on tolerant species.

The response of the game fish metric suggests a possible economic impact on the harvestable portion of stream fish assemblages resulting from mining. Game species comprising the game fish metric have varied biological and ecological traits, making it less likely that these fishes would respond to any single disturbance resulting from mining. However, even with the numerous life histories and species variation between US states that this metric characterizes, we found a negative response of game fishes to mining in all three ecoregions at low levels of mine density, primarily at the network scale, suggesting that upstream mines degrade downstream fisheries. Fisheries managers should consider this result when stocking fishes in catchments with mining.

Percent of herbivores was the only metric that increased with mine density, but only in SAP, and only for the mineral mines and minor coal mines classes in network catchments. In contrast, this metric decreased with increasing mines in NAP, and in TPL,
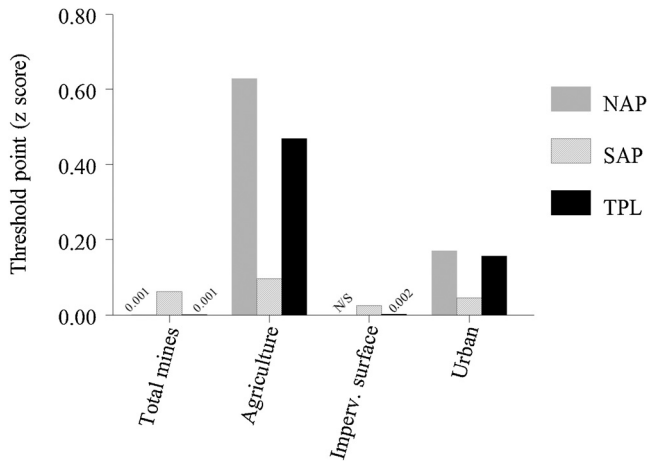
Fig. 4. NAP, SAP and TPL ecoregions comparison of z score transformed fish threshold responses for total mines, agricultural land use, impervious surface cover and urban land use. NAP comparison of intolerant individuals metric ($n = 7816$), SAP comparison of tolerant individuals metric ($n = 7619$) and TPL comparison of game species metric ($n = 6355$). Thresholds shown are from piecewise linear regression. Impervious surface for NAP was non-significant (N/S).

herbivores showed no significant threshold response with any of the mine classes. In SAP, this may be due to the composition of the metric. In SAP, herbivorous fishes make up 71% of the fish assemblage in reaches with mines, compared to the 31% in the NAP and 19% in the TPL. The herbivore metric in SAP is comprised of 148 species (Frimpong and Angermeier, 2009) which includes 86.7% of the species from the tolerant metric (86.7%). The same herbivore metric includes 93 species in NAP and 109 species in the TPL and both ecoregions have lower abundances of tolerant species. Alternatively, higher abundances of herbivores could be related to possible food web alteration within streams with mined catchments. Alteration of landscapes in mined catchments may have shifted the base of the food web from allochthonous to autochthonous sources (Schlosser, 1982) by loss of woody riparian canopy cover (Hill et al., 1995; US EPA, 2011) and higher nitrogen concentrations (Word, 2007) and in turn increased primary production (Allan, 2004).

4.2. Comparison with other anthropogenic disturbances

Mine activities occurring on the landscape may have similar general disturbances to stream fish habitats as agriculture, urban land use and impervious surfaces, but they may also have unique disturbances that may be more detrimental to streams than other anthropogenic land uses. The responses of intolerant individuals in NAP, tolerant individuals in SAP and game fish in TPL to the total number of mines was often lower and less variable than the responses to urban and agriculture land uses. The very low threshold responses to the total number of mines in NAP and TPL consists of a single mine in a 1000 km² watershed, that equates to 62 and 60% of catchments with mines, respectively. The exception was SAP which had low threshold responses by the tolerant metric for all disturbance classes.

Allan (2004) suggested that agricultural land use can have a weak positive influence on some fish metrics at low intensities. The NAP intolerant metric and TPL game metric did not respond to agricultural land use until over half the watershed was classified as agriculture. Those same metrics responded to urban land use with thresholds around 17 and 16% of the catchment, respectively. This agrees with the conclusion of Wang et al. (1997) work in Wisconsin streams that urban land use can reach 20% before fish assemblages respond. The fish metrics' response to impervious surface cover

was most similar to the total mines class, on the low end of the z scores gradient, similar to Ladson et al. (2006) where >2% impervious surface cover lead to alteration of the fish community. Paul and Meyer's (2001) review of streams in urban landscapes conclusion that amount of impervious surface in a catchment was a good predictor of the stream's biotic integrity; the density of mines within a catchment could be interpreted the same way.

4.3. Importance of scale

Our fish metrics displayed similar responses to mining as others in the literature (Letterman and Mitsch, 1978; Howells et al., 1983; Ferreri et al., 2004; Schorr and Backer, 2006; US EPA, 2011), but we demonstrated that mine influences are more pronounced at larger spatial scales than previously tested. In the conterminous United States (US), every state has mining within its borders. Coal or mineral mines occur in 2.7% of local streams catchments and 10.8% of network catchments, excluding great rivers. This is based on our mine data which, is incomplete but the most comprehensive available. Land use disturbance may be best expressed on the entire fish assemblage at larger spatial scales (Schlosser and Angermeier, 1995; Lammert and Allan, 1999; Hitt and Angermeier, 2011). Disturbance from mines may be underestimated when only looking at local spatial scales. In all ecoregions, threshold responses were the strongest (most significant thresholds) at the network catchment scale. Mine impacts may be disproportionate to the area they encompass, because a few mines have the potential to cause very low threshold points. The numerous network catchment threshold responses suggest a cumulative effect of mines as a regional source of disturbance. As mine density increases, and associated disturbances accumulate downstream, fish assemblages respond with negative wedge-shaped declines in abundance.

5. Conclusion

Fish assemblage threshold responses to mining were detected in three large ecoregions and through use of thousands of samples, indicating that mining may have negative effects on assemblage diversity and evenness, numbers of game species, as well as numbers of species with specific life history strategies, habitat preferences and trophic ecologies. Fish metric threshold responses detected in this studied occurred with relatively low densities of mines in stream catchments. For example, a single mine in a medium-sized river basin (>1000 km²) has the potential to alter fish assemblage in the stream draining that catchment. We acknowledge that our analyses were based on a landscape approach associating relationships between stream fishes and mines in catchments over broad spatial extents, however, repeatability in the trends detected with multiple fish metrics and for multiple classes of mines lend credence to our conclusions. This study underscores the fact that mines can in fact act as a regional source of stress to stream fishes, similar to urban land use and agriculture, and also emphasizes the importance of conducting more research to identify direct and indirect ways that mines may be influencing stream fishes. With new GIS landscape data layers, like the USGS coal layer, better opportunities exist to improve modeling of specific mining influences on stream systems.

There is also an opportunity for agencies and managers to consider the landscape influence of mining and improve fisheries habitat through actions including restoration, mitigation and preservation. When monitoring mined catchments, managers should include baseline ecological and environmental research not only at locally impacted reaches but at downstream reaches to account for the network alteration to fish assemblages. Mining should be excluded from ecologically- and culturally-significant

catchments, since we did not detect negative fish assemblage responses in only the largest size classes of rivers with low densities of mines. When managing for game fish in streams, managers should consider landscape scale influences of mines upstream, because mines at low densities have the potential to negatively impact downstream fisheries. Furthermore, the US has the world's largest estimated recoverable reserves of coal, and production will increase over the next two decades (US EIA, 2012), suggesting that alteration of stream fish assemblages may intensify in the future.

Acknowledgments

Appendix A.

Tolerant species listed in order by scientific name. Citation abbreviations: O, Ohio EPA, 1987; J, Jester et al., 1992; L, Lyons, 1992; W, Whittier and Hughes, 1998; B, Barbour et al., 1999; H, Halliwell et al., 1999; P, Pirhalla, 2004.

Common name	Scientific name	Cited by
Black bullhead	*Ameiurus melas*	J
Yellow bullhead	*Ameiurus natalis*	O, J, L, B, H, P
Brown bullhead	*Ameiurus nebulosus*	O, W, B, H, P
River carpsucker	*Carpiodes carpio*	J
White sucker	*Catostomus commersonii*	O, L, B, H
Common carp	*Cyprinus carpio*	O, J, L, W, B, H
Banded killifish	*Fundulus diaphanus*	B, H, P
Mummichog	*Fundulus heteroclitus*	W, H, P
Green sunfish	*Lepomis cyanellus*	O, J, L, B, H
Bluegill	*Lepomis macrochirus*	W, H, P
Yellow perch	*Perca flavescens*	J, P
Bluntnose minnow	*Pimephales notatus*	O, L, B, H

(Continued)

Common name	Scientific name	Cited by
Fathead minnow	*Pimephales promelas*	O, J, L, B, H, P
Eastern blacknose dace	*Rhinichthys atratulus*	O, L, B, H, P
Creek chub	*Semotilus atromaculatus*	O, L, B, H, P

Appendix B.

Intolerant species listed in order by scientific name. Citation abbreviations: O, Ohio EPA, 1987; J, Jester, et al. 1992; L, Lyons, 1992; W, Whittier and Hughes, 1998; B, Barbour et al., 1999; H, Halliwell et al., 1999; P, Pirhalla, 2004.

Common name	Scientific name	Cited by
Shortnose sturgeon	*Acipenser brevirostrum*	B
Atlantic sturgeon	*Acipenser oxyrinchus*	B
Eastern sand darter	*Ammocrypta pellucida*	B, H
Flier	*Centrarchus macropterus*	J
Redside dace	*Clinostomus elongatus*	O, B, H
Mottled sculpin	*Cottus bairdii*	O, L, B, H
Banded sculpin	*Cottus carolinae*	J
Banded pygmy sunfish	*Elassoma zonatum*	J
Blackbanded sunfish	*Enneacanthus chaetodon*	B, H
Streamline chub	*Erimystax dissimilis*	B, H
Gravel chub	*Erimystax x-punctatus*	J, H
Bluebreast darter	*Etheostoma camurum*	B, H
Harlequin darter	*Etheostoma histrio*	J, B
Spotted darter	*Etheostoma maculatum*	B, H
Banded darter	*Etheostoma zonale*	J, L, B, H
Northern studfish	*Fundulus catenatus*	J, B
Bigeye chub	*Hybopsis amblops*	O, J, B
Northern hog sucker	*Hypentelium nigricans*	O, J, L, B, P
Northern brook lamprey	*Ichthyomyzon fossor*	B, H
Southern brook lamprey	*Ichthyomyzon gagei*	J, B
Mountain brook lamprey	*Ichthyomyzon greeleyi*	B, H
American brook lamprey	*Lampetra appendix*	B, H
River redhorse	*Moxostoma carinatum*	O, J, B, H
Black redhorse	*Moxostoma cervinum*	O, B, H
Greater jumprock	*Moxostoma lachneri*	B
Greater redhorse	*Moxostoma valenciennesi*	L, B, H
Hornyhead chub	*Nocomis biguttatus*	O, B
River chub	*Nocomis micropogon*	O, B, P
Rosyface shiner	*Notropis rubellus*	O, J, L, B, H, P
Mountain madtom	*Noturus eleutherus*	J, B
Slender madtom	*Noturus exilis*	J, B
Stonecat	*Noturus flavus*	O, J, B
Brindled madtom	*Noturus miurus*	O, J, B
Cutthroat trout	*Oncorhynchus clarki*	B
Rainbow trout	*Oncorhynchus mykiss*	J, N
Gilt darter	*Percina evides*	B, H
Southern redbelly dace	*Phoxinus erythrogaster*	J
Atlantic salmon	*Salmo salar*	W, H
Dolly varden	*Salvelinus malma*	L, H
Lake trout	*Salvelinus namaycush*	L, H
Shovelnose sturgeon	*Scaphirhynchus platorynchus*	B

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