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Fish assemblage–environment relationships suggest differential trophic responses to heavy metal contamination

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Abstract

1. While ecotoxicology has long recognised the importance of identifying levels at which contaminants pose threats to biota, most estimates of species responses to toxicants are derived from controlled laboratory studies and may hold limited relevance to natural systems. However, designing appropriate field-based studies investigating contaminant induced changes in assemblages has been challenging, partially due to the difficulty in identifying comparable uncontaminated reference sites. The aim of this study is to characterise the effects of heavy metal contamination on natural fish assemblages using an ecologically relevant catchment-scale design. We hypothesise that environmental variables, including discharge, sediment, and landscape variables, can be used to characterise differences in fish species richness and abundances between sites contaminated with heavy metals and uncontaminated reference sites.
2. We apply a geographic information systems approach that uses assemblage–environment relationships developed using hydrologic model outputs, land cover, and topographic data from uncontaminated reference sites to predict expected fish species richness and abundance at sites contaminated with heavy-metals within the Big River catchment in south-eastern Missouri, U.S.A. These predicted levels of richness and abundance are then compared to observed assemblages at contaminated sites to estimate the potential impacts of historical lead mining activities on freshwater taxa.
3. We developed models that characterised variation in Centrarchidae (bass and sunfish) richness and abundance, Cyprinidae (minnows) abundance, and Percidae (darters) richness using variables including streamflow regime, suspended sediment concentration, and land cover at uncontaminated sites. Using these relationships, we predicted expected fish species richness and abundance at heavy metal contaminated sites across the Big River catchment and found a significant reduction in centrarchid abundance from field-collected data compared to predicted estimates.
4. Our results suggest that centrarchids, which tend to occupy a higher trophic level than cyprinids and percids, have lower abundances at sites contaminated with heavy metals than predicted by assemblage–environment relationships. These

decreases in abundance are not associated with decreases in centrarchid species richness, cyprinid abundance, or percid richness.
5. This geographic information systems-based approach provides a useful and ecologically relevant framework for understanding the response of taxa to the presence of contaminants without assuming habitat equivalence across sites. Our findings also suggest the need for further research regarding how heavy metals impact fishes of varying trophic levels in natural settings.

KEYWORDS
catchment, ecotoxicology, geographic information systems, hydrology, species distribution modelling

1 | INTRODUCTION

Freshwater ecosystems represent some of Earth's most threatened habitats. Consequently, developing a robust understanding of factors that may impact taxa in these systems and cause reductions in population sizes or local extirpations of species is critical (Dudgeon et al., 2006; Sala et al., 2000). One such threat to freshwater species is pollution from heavy metals, which have long been recognised to pose serious threats to freshwater systems due to the acute and chronic effects heavy metals can have on aquatic taxa (Boyd, 2010; Dixit et al., 2015; Prosi, 1981; Warnick & Bell, 1969).

Most studies of heavy metals in freshwater systems focus on identifying toxicity thresholds associated with altered behaviour and death in laboratory trials or identifying bioindicators of exposure in field-collected samples (Adams, 1995; Authman, Zaki, Khallaf, & Abbas, 2015; Hickie, Hutchinson, Dixon, & Hodson, 1993; Roesijadi, 1992; Schmitt et al., 2007; Vardy, Santore, Ryan, Giesy, & Hecker, 2014). This bias toward laboratory experiments and biomonitoring has generated criticisms for the lack of ecological relevance of the results of these studies, as they do not account for how species exposed to heavy metals will respond when they are subjected to other ecological pressures (competition, food acquisition, reproduction, etc.) (Cairns, 1992; Chapman, 2002; Filser, 2008; Forbes & Calow, 2002). To realise the full range of effects toxicants have on ecosystems, it is necessary to understand how exposure influences biota in natural environments. This approach can afford a more realistic perspective of the community-level effects of pollution and resulting alterations to ecosystems (Filser et al., 2008; Schmitt-Jansen, Veit, Dudel, & Altenburger, 2008).

Lead (Pb) mining can result in high levels of Pb contamination in freshwater systems as well as other associated heavy metals, including zinc (Zn), cadmium (Cd), and copper (Cu) (Gale, Adams, Wixson, Loftin, & Huang, 2002, 2004). These metals are known to bioaccumulate in the tissues of fishes, and laboratory studies have demonstrated that exposure to these pollutants can result in anaemia, decrease swimming performance, impede development and reproduction, and cause physical abnormalities (Atchison, Henry, & Sandheinrich, 1987; Authman et al., 2015; Pain, 1995). Although recent work has addressed the impacts of Pb-mining on natural fish and invertebrate populations by identifying reductions in species densities or richness in contaminated areas compared to non-contaminated reference sites (Allert et al., 2009, 2013; Maret & MacCoy, 2002; Maret, Cain, MacCoy, & Short, 2003), comparisons between contaminated and reference sites rarely account for variation in physical habitat between sites, or how this variation may influence species diversity and population sizes. Furthermore, a limited amount of research has specifically investigated how the effects of continued heavy metal exposure on fish species richness and abundance may vary among taxonomic and ecological (e.g. trophic) groups. Moreover, while recent studies have used geographic information systems (GIS) to characterise and predict fish assemblage responses to anthropogenic impacts at catchment or broader scales (e.g. Bailey, Linke, & Yates, 2014; Clarke, Furse, Wright, & Moss, 1996; De Zwart, Dyer, Posthuma, & Hawkins, 2006; Kapo, Burton, De Zwart, Posthuma, & Dyer, 2008; Knouft & Chu, 2015; Pletterbauer, Melcher, Ferreira, & Schmutz, 2015), this approach has yet to be applied at a catchment scale to assess the impacts of heavy metals at contaminated sites compared to uncontaminated reference sites using hydrologic model outputs and landscape variables.

The Big River catchment (BRC) lies within the Old Lead Belt of south-eastern Missouri and has high levels of heavy metal contamination due to historical Pb-mining (Pavlowsky, Owen, & Martin, 2010; Pavlowsky, Lecce, Owen, & Martin, 2017; Schmitt & Finger, 1982). Fishes within these contaminated areas contain high levels of heavy metals in their tissues (Czarnezki, 1985; Gale et al., 2002, 2004; Schmitt & McKee, 2016). The objectives of this study are to use catchment-scale environmental variables relevant to the distribution and abundance of fishes within the BRC, including streamflow, sediment, land cover, and topography, to predict fish species richness and abundance at uncontaminated sites within the BRC. We then use these assemblage–environment relationships to predict expected fish species richness and abundance within an area of known contamination from Pb-mining activities. These expected assemblages are then compared to the observed fish assemblages at contaminated sites to assess whether pollution from historical Pb-mining may be influencing species richness or abundance. To investigate the role of variation in heavy metal impacts among trophic levels, we focused our analyses on Centrarchidae (bass and sunfish), Cyprinidae (minnows), and Percidae (darters), as centrarchids represent the highest trophic level of fishes in the BRC, while cyprinids and percids represent the most abundant and diverse fish prey species in the BRC. We hypothesise that contaminated sites will be characterised by decreased species richness and abundance relative to predicted richness and abundance based on relationships at uncontaminated sites.

2 | METHODS

2.1 | Study area

The Big River is a primary tributary of the Meramec River, which lies in south-eastern Missouri, U.S.A. Land cover within the BRC is approximately 72% forested, 18% grassland, 7% developed, 1% agriculture, and 1% water (MODNR, 2013). One hundred of the approximately 200 species of fishes found in Missouri occur in the BRC (MDC, 1997; Pflieger, 1997). The BRC is considered highly impaired due to historical Pb-mining within the catchment, which began in the 1800s and concluded in the 1970s (Czarnezki, 1985; Gale et al., 2002, 2004; Pavlowsky et al., 2010, 2017; Schmitt & Finger, 1982). Erosion of chat and tailing piles as well as spillover from tailing ponds, have resulted in extensive contamination from heavy metals across the BRC, with nearly 160 km of streams within the catchment designated as impaired based on their exceedance of state water quality standards for Pb, Zn, and Cd (MODNR, 2010a,b). The mine and metallic mineral waste piles are located northeast of the Irondale United States Geological Survey (USGS) streamflow gauge, resulting in contamination along the remainder of the downstream portion of the Big River to its confluence with the Meramec River, as well as in Flat River Creek (Figure 1b). Environmental Protection Agency designation of the Big River Mine Tailings/St. Joe Minerals Corporation Superfund sites have included stabilisation, covering, and revegetation of the chat and tailings piles to prevent further erosion and sloughing into streams (EPA, 2011); however, there have been limited

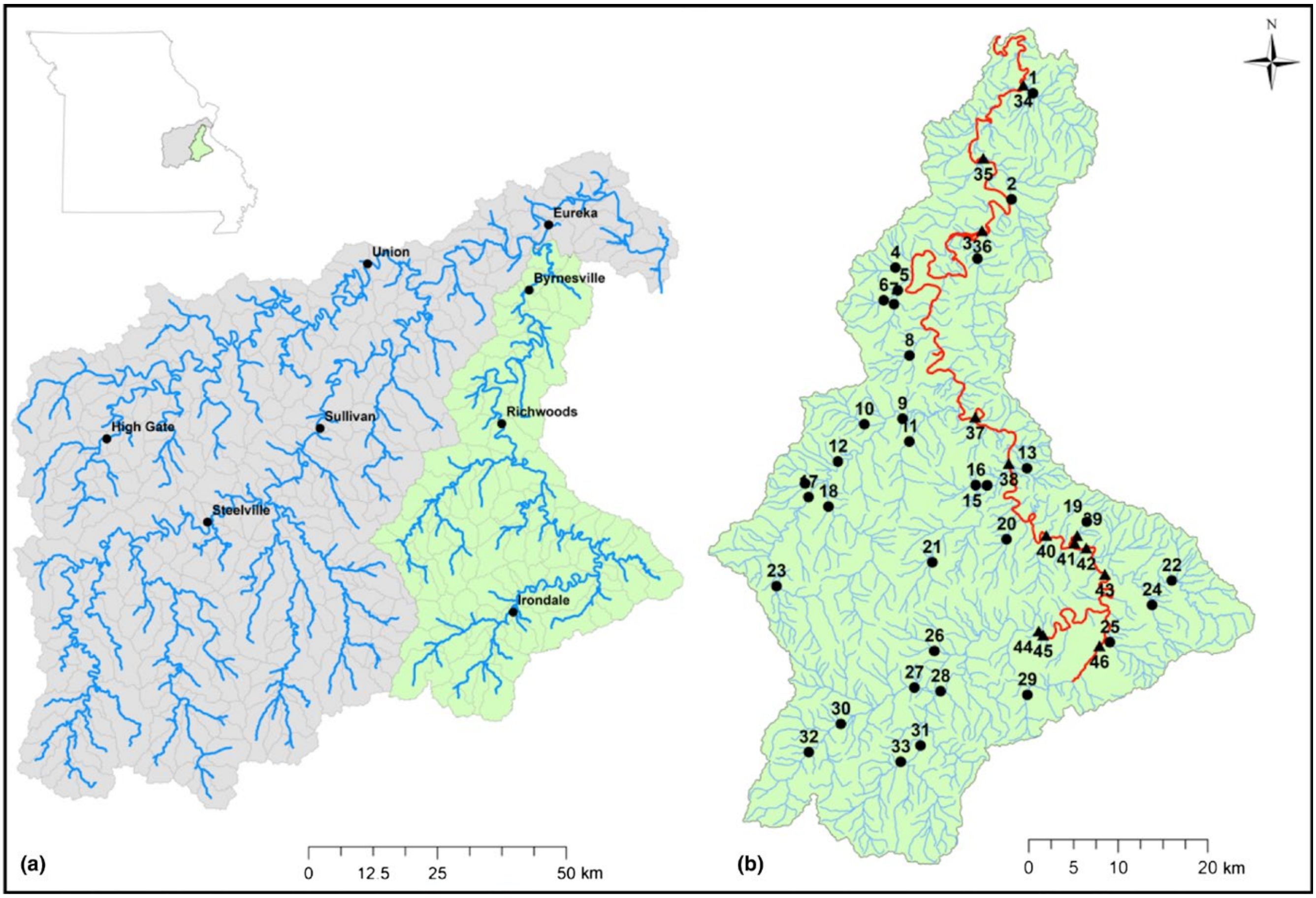

FIGURE 1 Map of the Meramec River Watershed (Missouri, U.S.A). The green shaded area represents the Big River catchment. (a) Grey outlines represent the sub-catchments where SWAT model estimates of streamflow and sediment regime were derived. Black points denote locations of the eight USGS streamflow gauges as well as the USGS suspended sediment gauge (Byrnesville). (b) Sections of the stream network labelled in red that are considered *impaired* due to exceedance of water quality standards for Pb, Zn, and Cd from Pb-mining waste contamination. Fish sampling sites are divided into those that do not exhibit heavy metal contamination (circles) and sites that are within the area of contamination (triangles) [Colour figure can be viewed at wileyonlinelibrary.com]

attempts to remediate the contamination already present in the streams and high levels of contaminants remain in these areas. These impaired areas are also under a fish consumption warning by the Missouri Department of Health and Senior Services due to increased heavy metal concentrations in the tissues of fishes inhabiting these stream reaches (MODNR, 2013).

2.2 | Fish assemblage data

Fishes were sampled across the BRC from 1984 to 2009 by the Missouri Department of Conservation using seining and electrofishing based on the standardised methodology of the Resource Assessment and Monitoring Program (Fischer & Combes, 2003) (Figure 1b). All sites were sampled via electrofishing once and seining up to twice to provide thorough coverage and standardise sampling efforts across sites of varying habitat composition. If samples were collected in multiple years at the same site, we used only the most recent year of sampling. We divided sites into non-contaminated and mining-contaminated sites based on whether the sampled location was within the water bodies considered impaired based on water quality standards (MODNR, 2010a,b). In total, we used fish data from 46 sites for analyses, 33 of which occurred within the non-contaminated portion of the catchment and 13 in contaminated areas. Fish species with inconclusive identifications across sampling locations were removed from the data set prior to analyses (these included *Campostoma anomalum*, *Campostoma oligolepis*, *Cyprinella spiloptera*, and *Cyprinella whipplei* of the family Cyprinidae). Hybrid individuals were also removed from the data set. Following removal of these records, we calculated species richness (total number of species at a location) as well as the total number of individuals across species (hereafter referred to as abundance) at each site for Centrarchidae, Cyprinidae, and Percidae (Supporting Information Table S1).

2.3 | Hydrologic modelling

We developed a Soil and Water Assessment Tool (SWAT) hydrologic model to estimate the streamflow (i.e. discharge) and sediment across the entire Meramec River Watershed (Figure 1a). SWAT is a semi-distributed, hydrologic model that can simulate the impacts of land cover and management on water and sediment in complex catchments. The major components of SWAT include climate, hydrology, sediment yields, land management practices, plant growth, nutrient loads, pesticides applications, and bacteria and pathogen dynamics (Arnold et al., 2012; Neitsch, Arnold, Kiniry, & Williams, 2011). A detailed description of the SWAT model and its varied applications can be found at the SWAT website (http://swat.tamu.edu/).

During the modelling process, a catchment is divided into multiple sub-catchments and each sub-catchment is composed of one or more hydrologic response units (HRUs) based on unique combinations of land cover, soil, and slope classifications. Water yields from HRUs are calculated using the water balance equation. Water and sediment loads (SLs) from each HRU are aggregated within the respective sub-catchment and then routed to the catchment outlet through the stream network (Arnold et al., 2012).

Soil and Water Assessment Tool model inputs include topographic, soil, land cover, and climate data. The digital elevation model (DEM) data, which represents the topographic surface for the Meramec River Watershed region, was downloaded from the USGS 3D Elevation Program (Sugarbaker et al., 2014). The DEM has a 1 arc-second resolution (approximately 30 m × 30 m) and was used for delineating the entire catchment and sub-catchments. By setting 1,200 m^2 as a threshold for the minimum sub-catchment area, a total of 470 sub-catchments and 1,409 HRUs were delineated. Land cover data at a 30-m resolution for the Meramec River Watershed were obtained from the Multi-Resolution Land Characteristics Consortium: National Land Cover Dataset 2011 (Homer et al., 2015). Soil data were downloaded from the United States Department of Agriculture Natural Resources Conservation Service Soil Survey Geographic Database (1:12,000 scale). Long-term precipitation (10 stations) and air temperature data (four stations) for the Meramec River Watershed were downloaded from the National Oceanic and Atmospheric Administration—National Climatic Data Center. The precipitation and air temperature data span the 1978–2014 period. Relative humidity, solar radiation, and wind speed data were simulated using the SWAT built-in weather generator.

The period of simulation is from 1 January 1978 to 31 December 2014. The first 3 years of simulation (1978–1980) were used as a warm-up period to obtain the initial conditions of the models. The model was calibrated using the observed streamflow data from eight USGS streamflow gauges distributed across the Meramec catchment while SL was calibrated to the suspended SL measured at a gauge located at the outlet of BRC at Byrnesville (Figure 1a). The calibration period for streamflow was January 1996–December 2012, while the validation periods were January 1981–December 1995 and January 2013–December 2014. The calibration and validation periods for SL were November 2011–December 2012 and January 2013–September 2013, respectively. Model simulations were conducted at a monthly time step.

The SWAT model uses many parameters in simulating hydrologic and sediment transport processes. In this study, an auto-calibration program, SWAT-CUP, was applied and the Sequential Uncertainty Fitting algorithm was used to perform model calibration, validation, and uncertainty analyses (Abbaspour, Johnson, & van Genuchten, 2004; Abbaspour et al., 2007). The statistical criteria used for model performance evaluation were the coefficient of determination (R^2) and the Nash–Sutcliffe coefficient (Nash & Sutcliffe, 1970).

2.4 | Environmental predictor variables

Streamflow, sediment concentration, land cover, and topography were selected as variables likely to influence fish assemblages across the BRC (Allan, 2004; Helms, Schoonover, & Feminella, 2009; Knouft & Chu, 2015; Niu, Franczyk, & Knouft, 2012; Park, Grenouillet, Esperance, & Lek, 2006; Poff & Allan, 1995; Poff et al., 1997; Sutherland, Meyer, & Gardiner, 2002; Waite & Carpenter, 2000;

Walters, Roy, & Leigh, 2009). The SWAT model-derived monthly estimates of streamflow and sediment for each sub-catchment across the BRC were used to develop several metrics that potentially drive the assemblage–environment relationships. From these monthly estimates, we calculated the average annual streamflow ($Flow_{avg}$, m^3/s), maximum annual streamflow ($Flow_{max}$, m^3/s), minimum annual streamflow ($Flow_{min}$, m^3/s), and intra-annual coefficient of variation in streamflow ($Flow_{var}$), which quantifies intra-annual streamflow variability. $Flow_{avg}$ was calculated as the average across the 12 monthly averages for the year. $Flow_{max}$ and $Flow_{min}$ represent the highest and lowest monthly average stream-flows within the year, respectively. $Flow_{var}$ represents the standard deviation of monthly streamflow averages divided by the average of all monthly averages for the year. Each of these metrics was paired with the fish sampling locations using the hydrologic data from the year prior to when fish were collected.

Sediment regime within each sub-catchment was computed using SWAT model derived monthly estimates of total suspended sediment (TSS; mg/L) and monthly sums of total SL (tons) for the year in which the fish sample was collected at each site. The metric used to characterise the TSS at each site was the average annual TSS, which was calculated as the average across the monthly estimates within each year. SL at each site was characterised by the sum of SL across the year. Sediment data from the year prior to fish collection at each site were used in subsequent assemblage–environment relationships.

Land cover for each site was characterised as the percentage of forested land cover within the sub-catchment containing each site. To calculate this variable, we used 2006 land cover data (30-m resolution) for the catchment and the Zonal Tabulate Area tool in ArcGIS 10.4.1 to determine the summed area of forested land cover (including deciduous, evergreen and mixed forest) and divided the sum by the total area of each sub-catchment (Fry et al., 2011). The use of forested land cover allows for the application of a single variable with ecological relevance to in-stream habitat (Meador & Goldstein, 2003). Topography was represented by the slope at each site using the same DEM for the Meramec River Watershed region that was used in hydrologic model development (Sugarbaker et al., 2014). Slope was used to characterise how quickly water moved through a stream reach. All spatial data organisation and analyses were conducted in ArcGIS 10.4.1 using the NAD 1983 UTM Zone 15N projected coordinate system.

2.5 | Data analyses

We used data from non-contaminated sites to characterise the relationship between the selected environmental variables (streamflow, sediment, land cover, and slope) and species richness and abundance across the BRC. To meet assumptions of normality for linear regression, percent forested land cover was logit transformed, and $\log_{10}$ transformations were applied to $Flow_{avg}$, $Flow_{max}$, $Flow_{min}$, $Flow_{var}$, SL, and slope. $Flow_{avg}$, $Flow_{max}$, and $Flow_{min}$ were highly correlated ($r > 0.88$). As a result, we conducted a principal component analysis (PCA) to encapsulate the variation explained by these streamflow metrics into uncorrelated principal component (PC) scores. PCs that characterised up to a total of 90% of the variation in $Flow_{avg}$, $Flow_{max}$, and $Flow_{min}$ along with the normalised $Flow_{var}$, TSS, SL, land cover, and slope metrics were used as predictor variables in multiple regressions describing variation in species richness and abundance across the non-contaminated sites within the BRC. To minimise the total number of predictor variables in these models, only linear relationships were assessed. Optimal predictive models were selected using forward stepwise regression with Akaike's information criterion corrected for small sample size (AICc) to select the model that explained the greatest variation in the assemblage metric with the fewest variables. Variables were retained in the model based on the largest improvement to AICc (Burnham & Anderson, 2004). The final model retained had ΔAICc > 2 from the model with the next lowest AICc.

Centrarchid species generally become piscivorous throughout their lives and tend to feed at higher trophic levels compared to insectivorous cyprinids and percids. To characterise the differing relationships exhibited by species feeding at different trophic levels, separate regression analyses were conducted to investigate the relationships between the environmental variables and species richness and abundances among Centrarchidae, Cyprinidae, and Percidae. Sample sites with zero observations for a family were not used in model development.

When environmental variables predicted variation in richness or abundance at uncontaminated sites, we used these relationships to predict richness and abundance at contaminated sites. We assessed the potential impacts of contamination from Pb-mining on fish assemblages by determining the difference between the predicted and observed fish species richness and abundance at each contaminated site using paired *t* tests with a Bonferroni correction for multiple comparisons. The effect sizes of all significant differences between the expected and observed communities were determined using Cohen's D (Cohen, 1992). Statistical analyses were conducted using R 3.2.4 (R Development Core Team, 2017).

3 | RESULTS

3.1 | Hydrologic modelling

The timing of occurrence for both low and peak flows predicted by the SWAT model was generally in accordance with observed data (Supporting Information Figure S1). The R^2 values indicate a strong correlation between the observed and simulated streamflow during both calibration and validation periods (Supporting Information Figure S1, Table 1). During calibration, the model's performance simulating streamflow was *very good* (NSE > 0.75) at all streamflow gauges based on the performance rating developed by Moriasi et al. (2007), except for the gauge at High Gate, which was still considered *good* (0.65 < NSE ≤ 0.75). Values of NSE were 0.91 and 0.37 in sediment simulation for the calibration and validation periods, respectively (Supporting Information Figure S2, Table 1). The R^2 value for calibration indicated a strong correlation between observed and simulated SL.

3.2 | Assemblage–environment relationships

Principal component analysis of $Flow_{avg}$, $Flow_{max}$, and $Flow_{min}$ for the year of sampling revealed that the first principal component explained 95% of the variation across these flow metrics. Therefore, only the first principal component (hereafter referred to as $Flow_{PC}$) was retained for analyses. $Flow_{avg}$, $Flow_{max}$, and $Flow_{min}$ had factor loadings of −0.586, −0.585, and −0.561, respectively. As the sign of factor loadings and PC scores are arbitrary, for ease of interpretation, the signs of factor loadings and PC scores were reversed prior to regression analyses.

Results from stepwise regression based on AICc scores indicated that environmental variables were useful for predicting variation in fish assemblages across non-contaminated sites within the BRC for Centrarchidae richness and abundance, Cyprinidae abundance, and Percidae richness (Table 2). These relationships exhibited R^2 values ranging from 0.133 to 0.320 (Table 2). The variables included in the best models and the direction of relationships varied among families (Table 2). $Flow_{PC}$ explained substantial variation in both Centrarchidae richness and abundance, exhibiting positive relationships in both cases. The retained model for Centrarchidae richness also included a positive relationship with percent forested land cover. Cyprinidae abundance was best predicted by percent forested land cover as well, although the direction of the relationship was negative. Percidae richness exhibited a positive relationship with TSS. No environmental metrics were better than a random model at predicting Cyprinidae richness or Percidae abundance.

3.3 | Associations with contamination

There was a significant difference between observed and expected Centrarchidae abundance ($df = 12$, $t = 3.402$, $p = 0.005$) at contaminated sites, with the observed abundance being almost half of

TABLE 1 Model performance statistics, the coefficient of determination (R^2) and Nash–Sutcliffe coefficient (NSE), of streamflow and sediment load simulation

	Streamflow				Sediment load			
	Calibration		Validation		Calibration		Validation	
Station	R^2	NSE	R^2	NSE	R^2	NSE	R^2	NSE
Byrnesville	0.81	0.76	0.81	0.81	0.91	0.91	0.53	0.37
Richwoods	0.83	0.80	0.79	0.79				
Irondale	0.80	0.78	0.71	0.70				
Union	0.80	0.77	0.81	0.77				
High Gate	0.81	0.74	0.87	0.80				
Eureka	0.88	0.88	0.89	0.89				
Sullivan	0.84	0.76	0.88	0.86				
Steelville	0.82	0.69	0.86	0.81				

TABLE 2 Results and model coefficients from forward stepwise selection using Akaike's information criterion corrected for small sample size (AICc) to determine the relationship between environmental data and richness (Rich.) and abundance (Abund.) of Centrarchidae, Cyprinidae, and Percidae within the Big River catchment. The model presented for each diversity measure is the best model determine by having a ΔAICc > 2 from the model with the next lowest AICc score. Diversity metrics for which no model is presented indicates cases in which none of the candidate variables explain more variation than the mean

Taxonomic group	Number of sites	Measure	AICc	R^2	Variable	Coeff.	*SE*	*t*-value	*p*-value
Centrarchidae	32	Rich.	122.029	0.320	Intercept	3.935	0.428	9.189	<0.0001
					$Flow_{PC}$	0.653	0.208	-3.139	0.0039
					Forest LC	0.667	0.302	2.211	0.0351
		Abund.	353.743	0.179	Intercept	75.882	11.405	6.653	<0.0001
					$Flow_{PC}$	20.243	7.927	-2.554	0.0160
Cyprinidae	32	Rich.	—	—	—	—	—	—	—
		Abund.	427.841	0.133	Intercept	344.590	53.160	6.482	<0.0001
					Forest LC	−83.250	38.770	−2.147	0.0400
Percidae	33	Rich.	108.500	0.141	Intercept	3.716	0.232	16.020	<0.0001
					TSS	0.030	0.013	2.253	0.0315
		Abund.	—	—	—	—	—	—	—

the expected abundance, on average (Figure 2). The effect size of this difference is large based on the Cohen's *D* value of 0.944. No significant differences were found among observed and expected Centrarchidae richness (df = 12, t = 1.109, p = 0.289), Percidae richness (df = 9, t = −2.375, p = 0.042), and Cyprinidae abundance (df = 9, t = 0.731, p = 0.4836) based on Bonferroni corrections (α' = 0.05/number of tests; Figure 2).

4 | DISCUSSION

Prior research focused on the responses of aquatic assemblages to heavy metals has identified reductions in population densities or species richness in contaminated areas compared to uncontaminated reference sites (Allert et al., 2009, 2013; Maret & MacCoy, 2002; Maret et al., 2003). While typically these studies quantify physical habitat characteristics other than the presence of contaminants among reference and contaminated sites, they attribute taxonomic differences among sites to the contaminant alone, for at least two reasons. First, no evidence is found for significant differences in the environmental variables among sites, and therefore contaminated and uncontaminated sites are assumed to be ecologically equivalent (e.g. Maret et al., 2003). In other cases, significant differences are found among reference and contaminated sites, although the response in community composition is more strongly associated with the presence of contaminants (Allert et al., 2009, 2013; Maret & MacCoy, 2002). In both cases, these studies assume that differences in species assemblages are only due to the presence of contaminants, rather than also accounting for habitat differences between contaminated and uncontaminated sites. We avoid this assumption by using relationships between environmental characteristics and stream fish assemblages throughout the catchment to estimate the fish assemblages that would be expected in the absence of the existing contamination. We then determined the effects of contamination based on deviations from the expected assemblages.

Results suggest that when determining the relationships between environmental characteristics and fish species assemblages in the BRC, the most important predictor variables are related to streamflow, sedimentation, and surrounding land cover. $Flow_{PC}$ is positively associated with centrarchid richness and abundance, which suggests that as streamflow volume and variability increase across the catchment, so does the richness and abundance of centrarchids.

We found that the prevalence of forested landcover was an important predictor in our models; however, the direction of the relationship between percent of forested land cover and fish assemblages differed between taxonomic groups, with centrarchid richness being positively related to forested land cover and cyprinid abundance being higher at sites with lower proportions of forested land cover. These results, when considered with other literature investigating this relationship (e.g. Jones, Helfman, Harper, & Bolstad, 1999; Roth, Allan, & Erickson, 1996; Wang, Lyons, Kanehl, & Gatti, 1997; Walters et al., 2009) suggest that this relationship warrants further investigation given that responses often vary depending on response metric used to measure the fish assemblage (e.g. IBI or abundances) and among taxonomic groups.

The finding that percid richness is positively associated with TSS is not typically what would be expected given the generally negative effects of suspended sediment on fishes (Kemp, Sear, Collins, Naden, & Jones, 2011). Given the small magnitude of effect that TSS exhibited on percid richness (Table 2) and generally low levels of TSS across sites, subtle differences in unmeasured habitat characteristics associated with greater TSS may also be responsible for higher percid richness. Furthermore, stream-dwelling percid species occur in a variety of habitats and at sites with heterogeneous environmental conditions that can support a broader array of species (Pflieger, 1997).

Discharge, sediment, and landcover represent a fraction of the physical and biological environment in lotic ecosystems. The limited amount of variation (R^2) explained by our assemblage–environment relationship models is not surprising and suggests the role of additional environmental characteristics in regulating local fish abundance, particularly the amount of microhabitat (riffles, runs, and pools) available at a site (Knouft et al., 2011). Biological interactions are also important in structuring stream fish assemblages and information on these interactions would probably have added information to our predictions.

By determining contamination effects via differences between observed and expected assemblages, we demonstrated a negative response of centrarchid abundance to contamination, but no significant responses among centrarchid richness, percid richness, or cyprinid abundance. Species belonging to the family Centrarchidae

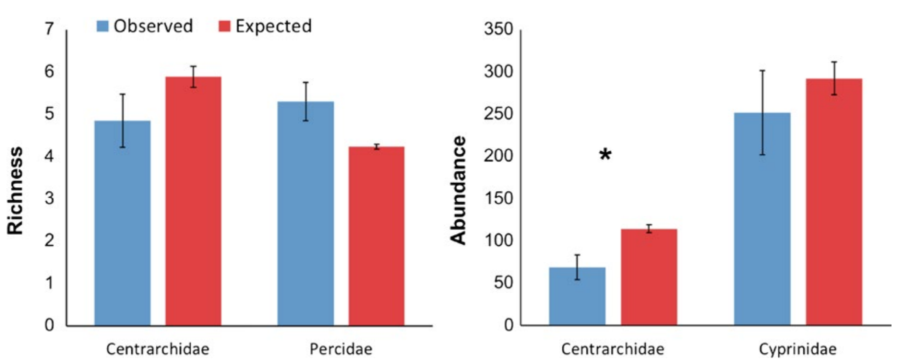

FIGURE 2 Results from comparisons of predicted species assemblages at Pb-mining contaminated sites based on environment-assemblage models and observed species assemblages at contaminated sites. Asterisk represents significant difference between groups. Error bars represent standard error [Colour figure can be viewed at wileyonlinelibrary.com]

often feed on a broader array of prey and at a higher trophic level when mature compared to cyprinids and percids (all cyprinids and percids in this study are insectivorous). Most centrarchid species consume other fishes when mature, potentially impacting prey population sizes (Knouft, 2002). Results did not suggest a reduction in the abundance of cyprinids at contaminated sites; thus, the decrease in abundance of centrarchids does not appear to be due to a lack in abundance of cyprinid prey or a lack in diversity of percid prey (Scalet, 1977). The decrease in centrarchid abundance is also not due to a reduction in centrarchid species richness. Furthermore, increased effects of heavy metal exposure on higher trophic level fishes may not be due to biomagnification as the heavy metals investigated are not known to biomagnify in the BRC (Cardwell, DeForest, Brix, & Adams, 2013).

Research on the effects of heavy metals on fishes of varying trophic levels has largely been limited to studies of differences in bioaccumulation rates and have demonstrated mixed results. Some studies from other regions with different species assemblages have demonstrated that various heavy metals are consistently present at higher concentrations within the tissues of piscivorous fishes (including fishes of the families Characidae and Ictaluridae) in comparison to fishes occupying lower trophic levels (Has-Schön et al., 2015; Jia, Wang, Qu, Wang, & Yang, 2017; Terra, Araujo, Calza, Lopes, & Teixeira, 2008). In contrast, studies that have investigated bioaccumulation rates among fishes within the BRC have found that some heavy metals, such as Pb, are typically higher in lower trophic level fishes in the family Catostomidae compared to centrarchids. However, this pattern is not apparent for other heavy metals with similar concentrations in fish tissues among trophic groups in the BRC (Gale et al., 2002, 2004; Schmitt & McKee, 2016). Therefore, it is difficult to determine whether these previous findings support or are in contrast to our findings of reduced abundance among higher trophic level fishes. This lack of consensus demonstrates a necessity for further studies of bioaccumulation rates among trophic levels and heavy metals. Furthermore, as bioaccumulation rates appear to vary among different metals, future research should investigate which metals are driving the deleterious effects on fish when they occur in mixtures, as is often the case in contaminated areas.

The relationships between heavy metal uptake, fitness, and performance among taxa can complicate the characterisation of variability in observed responses among groups and therefore, further research is required to dissect these relationships. For example, a potential route of further study could test whether poor swimming performance resulting from heavy metal exposure leads to reduced predation efficiency or feeding rates among piscivorous centrarchids (Atchison et al., 1987). Additionally, centrarchids are typically longer lived than cyprinids, which could lead to reduced abundances via increased lengths of exposure or altered age class distributions. There is also an apparent lack of published literature comparing heavy metal toxicity endpoints (e.g. LC50) among species belonging to the families investigated in this study. Expanding this area of research is also essential in order to determine how toxicity is correlated with trophic level or other differences among the species.

Our findings regarding differential responses of fish assemblages to Pb-mining contamination suggest potential alterations to the stream community across these sites due to decreases in centrarchid abundance. Decreases in predatory centrarchids have been suggested to influence the relationship between fish body size and population density as well as increased abundance among prey (Knouft, 2002; Mitchell & Knouft, 2009). Furthermore, the presence of centrarchids has been shown to have cascading ecosystem effects on algal abundances within streams (Power & Matthews, 1983; Power, Matthews, & Stewart, 1985).

Due to an incomplete understanding of the effects that contaminants exert on natural assemblages of species, there is a need to develop methodologies that can resolve these responses while maintaining ecological relevance. We demonstrate a potentially useful approach for examining assemblage level responses to contamination in natural systems by employing GIS-based analyses of assemblage–environment relationships driven by catchment scale environmental data. While this approach does not offer a mechanistic understanding of how contamination influences assemblage composition, it does provide ecologically relevant insights into the consequences of such contamination. By expanding our understanding of how effects of heavy metals and other contaminants of ecotoxicological concern scale from individual laboratory-based assessments to impacts on natural communities, we can improve our ability to determine levels at which these contaminants have ecological consequences.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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