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Examining the Impacts of Valley Fills in Stream Ecosystems on Amphibian and Aquatic Insect Communities in Southeastern Kentucky

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EXAMINING THE IMPACTS OF VALLEY FILLS IN STREAM ECOSYSTEMS ON AMPHIBIAN AND AQUATIC INSECT COMMUNITIES IN SOUTHEASTERN KENTUCKY.

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CHAPTER 1. VARIATION IN SALAMANDER AND AQUATIC INSECT COMMUNITIES AS IT RELATES TO STREAM CONDITION IN NATURAL AREAS OF SOUTHEASTERN KENTUCKY

AND

CHAPTER 2. EXAMINING THE IMPACTS OF VALLEY FILLS IN STREAM ECOSYSTEMS ON AMPHIBIAN AND AQUATIC INSECT COMMUNITIES IN SOUTHEASTERN KENTUCKY.

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Chapter 1. Abstract: Biodiversity is not evenly distributed, and understanding factors that determine spatial patterns of species diversity remains a key question in ecology. Because of their relatively high abundance and complex life cycles, stream salamanders and aquatic insects are important trophic links and serve a critical role in transferring energy. Despite this importance little research has examined their community structure simultaneously in aquatic ecosystems. The primary objective of this research was to determine the structure of these communities across natural areas of southeastern Kentucky and understand what factors impact their abundances and distributions. To address this, we sampled eight reference quality streams across the region, March–June 2014. Salamander sampling consisted of three sampling periods on a monthly basis, April–June 2014. Aquatic insect sampling consisted of a single sampling event in March 2014, with water and habitat sampling occurring during each aquatic insect and salamander sampling event. Within each stream, a 100-m reach was sampled for salamanders, aquatic insects, water quality, and habitat measurements. A principle component analysis (PCA) approach was used for factor reduction to create predictive models of environmental variables associated with salamander and aquatic insect abundance and richness. 390 salamanders (155 adult, 235 larvae; 7 species) and 1,163 aquatic insects (8 orders, 33 families) were sampled. Predictive models revealed associations between salamander and aquatic insect abundance and richness, presence and composition of cover objects, and stream pH and conductivity. Understanding patterns of community composition and distribution of aquatic insects and salamanders within reference quality aquatic ecosystems provides important information about

ecosystem functioning in undisturbed habitats in this region of high disturbance and anthropogenic land use.

Chapter 2. Abstract: Valley fills due to mountaintop-removal mining bury headwater streams and affect downstream water quality and ecological function. Past studies have focused on generally one taxonomic group or purely habitat and water quality affects. In this study we evaluated stream salamander and aquatic insect communities, metal concentrations in water and tissue, and stream quality and habitat in 10 streams affected by Valley fills (VFS) and 5 reference streams (RS) located in natural areas within 15 km of VFS. Within each stream, a 100-m reach was sampled for the above stated parameters. Salamander sampling consisted of three sampling periods on a monthly basis, April–June 2015. Aquatic insect sampling consisted of a single sampling event in March 2015, with water and habitat sampling occurring during each aquatic insect and salamander sampling event. This study captured 529 individual salamanders of eight species, with captures in RS (n=335) higher than in sampled VFS (n=194). A total of 1,034 aquatic insects representing 8 orders and 37 families were collected, and captures were higher for RS (n=597) than VF (n=447). Abundance, richness, and other community metrics of sampled salamander and aquatic insects were significantly higher in RS than VFS. Several habitat and environmental factors significantly differed between treatments including % silt, conductivity, selenium concentration in water and tissue, and canopy closure likely leading to the reduced communities of salamanders and aquatic insects observed. By approaching the issue of stream health through multiple abiotic factors and taxa, this study provides critical information of the effects of valley fills on stream quality and function.

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CHAPTER 1. VARIATION IN SALAMANDER AND AQUATIC INSECT COMMUNITIES AS IT RELATES TO STREAM CONDITION IN NATURAL AREAS OF SOUTHEASTERN KENTUCKY

INTRODUCTION

Biodiversity is not evenly distributed, and understanding factors that determine spatial patterns of species diversity remains a key question in ecology (Gaston, 2000). In stream ecosystems the distribution of organisms is a result of complex interactions including competition, shifts in habitat suitability and availability, and interactions of biotic and abiotic factors (Torgersen et al., 1999; Doi and Katano, 2008; Yeiser and Richter, 2015). Stream community composition is largely determined by the organization and dynamics of the physical stream habitat and the species available for colonizing an area (Wevers and Warren 1986; Frissell et al., 1986). Therefore, the locality of a stream and natural variability in biotic and abiotic factors within a stream likely influence the abundance and presence of stream taxa (Frissell et al., 1986; Doi and Katano, 2008; Yeiser and Richter, 2015).

The Central Appalachian Mountains is an ecoregion recognized as a global hotspot for aquatic biodiversity and endemism and is recognized as the global diversity hotspot for salamander species (Stein et al., 2000). However, this diversity is threatened by surface coal mining, which has become one of the dominant drivers of human land-use change in this region (Bernhardt and Palmer, 2011; Wood and Williams, 2013; Muncy et al., 2014). Despite the high human land-use in this region, a number of protected natural areas owned by federal, state, and non-governmental organizations exist and provide a refuge for this region's diversity (Abernathy et al., 2010). In order to understand the effects of anthropogenic change to stream ecosystems and conserve sensitive biota, research is needed to understand variation and diversity present in stream communities across natural areas in this landscape.

In Appalachian aquatic ecosystems, salamanders perform many key ecological functions (Marcot and Vander Hayden, 2001; Davic and Welsh, 2004). In terms of abundance and biomass, salamanders are often the dominant vertebrate predators in aquatic and terrestrial systems (Burton and Likens, 1975; Davic and Welsh, 2004). Because of their relatively high abundance and complex life cycles, salamanders are important links between invertebrate and vertebrate communities and serve a critical role in transferring energy between terrestrial and aquatic ecosystems (Petranka, 1998; Davic and Welsh, 2004; Hopkins, 2007).

Stream insect diversity is particularly high in headwater streams (Stout and Wallace, 2005; Clarke et al., 2008). Stream insects serve many functions in aquatic ecosystems, including regulation of nutrients via breakdown of organic material by shredder and decomposer feeding guilds, and impact levels of decomposition, productivity, and translocation of material within stream systems (Wallace and Webster, 1996). Stream insects also serve as a major prey base in aquatic ecosystem food webs (Pond et al., 2008), and specifically represent the major source of food for aquatic amphibians in stream ecosystems (Jackson et al., 2007). Therefore, determining factors that affect abundance and presence of these two taxa is important for understanding ecosystem processes (Reice, 1991; Petranka, 1998; Davic and Welsh, 2004; Pond, 2010).

The objective of this research was to determine the natural variation, community composition, and structure of salamander and aquatic insect communities in reference quality headwater streams across southeastern Kentucky, and which habitat and environmental variables best predict for their abundance and diversity. It was predicted that changes in community composition across the landscape will primarily be a result of differences in stream characteristics. It was also predicted that diversity of aquatic insects and salamanders will be high and covary across the landscape, based on their connected role in the trophic food web and due to similar habitat requirements.

METHODS

Study Area

Eight reference streams located in national and state protected areas throughout southeastern Kentucky were sampled in March–June 2014 in order to determine natural variation in salamander and aquatic insect communities across the region. These sites were considered reference quality streams with no mining history and within relatively unaltered watersheds. Reference stream sites consisted of mature, forested first-order headwater streams considered to be some of the best quality headwater streams in the region based on discussions with personnel from the Kentucky Division of Forestry, Kentucky Division of Water, Kentucky State Nature Preserves, and Kentucky Natural Lands Trust. The forest stands were at least 70 years old, including old-growth forest, and the headwaters of the streams and sampled stream reaches were within national and state protected area boundaries. These protected areas are located north of Pine

Mountain and on the north and south side of Black and Cumberland mountains in Bell, Harlan, and Letcher counties (Fig. 1).

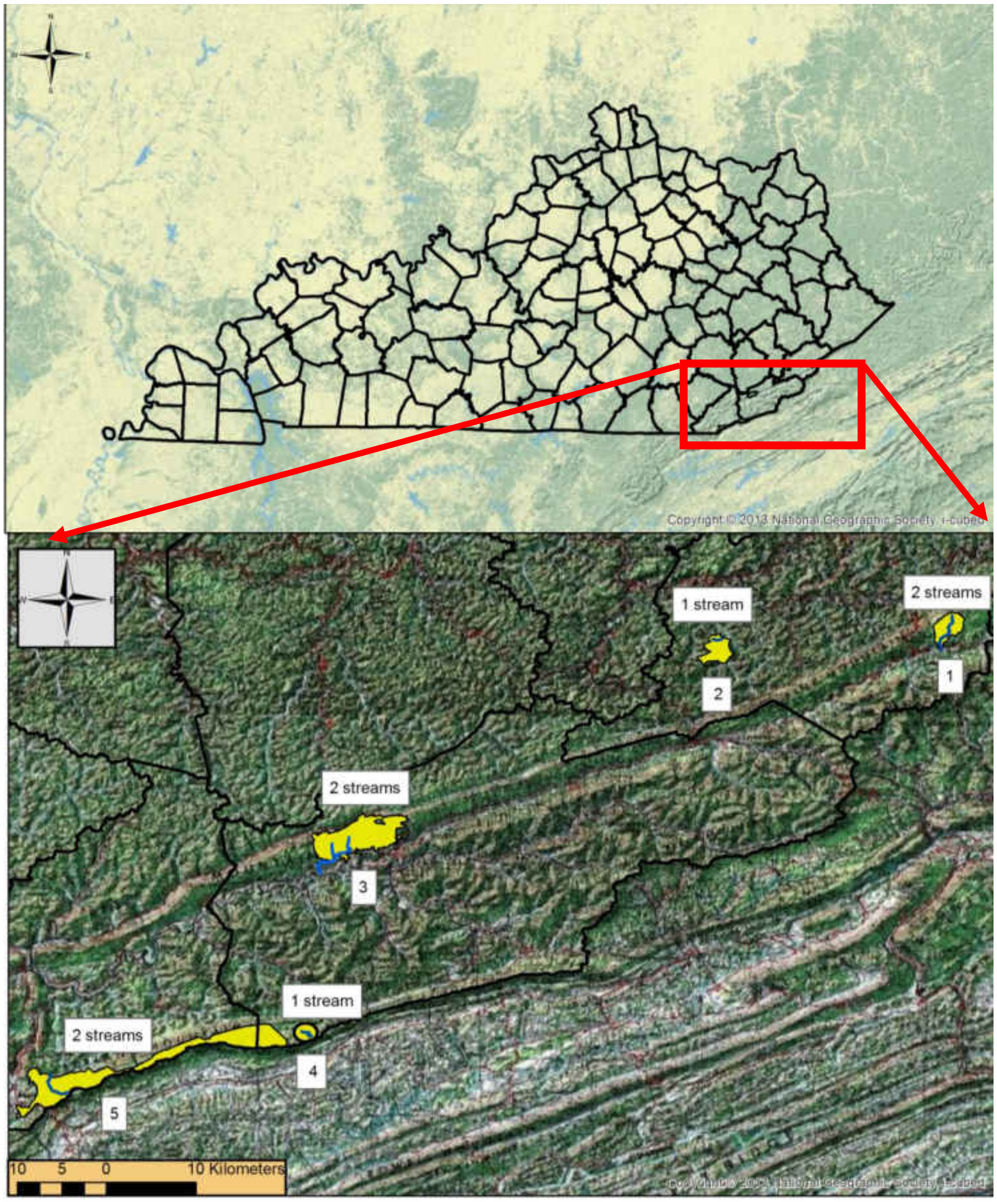

Figure 1. Research study sites (March–June of 2014) in southeastern Kentucky. Moving from east to west sites include Bad Branch State Nature Preserves (1), Lilley Cornett Woods(2), Blanton Forest State Nature Preserve (3), Martin's Fork Wildlife Management Area (4), and Cumberland Gap National Historic Park (5). Numbers above each sample site refers to the number of streams sampled per locality.

Study Design

Within each stream, a 100-m reach was positioned 20 meters above the confluence of each headwater stream to a larger stream, to standardize sampling and to decrease the likelihood of the stream drying. Habitat sampling occurred four times in 1-month intervals in spring (March–June) 2014. Salamander sampling consisted of three sampling periods in 1-month intervals, April–June 2014, while aquatic insect sampling consisted of a single sampling event in March 2014. All sites were sampled within four days of one another per sampling event and at least 48 hours since the last precipitation event. The order of sampling between reference streams was randomized within each sampling event to avoid temporal bias.

Habitat Sampling

The dominant mesohabitat, cover types, canopy closure, water depth (cm), stream width (m), and water temperature were measured at three sampling points corresponding to the upper, middle, and lower points of each reach (i.e. at 0, 50, and 100 m) per sampling event. At each point, the proportion of dominant mesohabitat types (run, riffle, and pool) and cover types (silt, sand, gravel, pebble, cobble, boulder, muck, and detritus) were estimated based on a view looking directly down upon the stream (Jung, 2002; Wood and Williams, 2013). The amount of canopy closure was visually estimated using a spherical densiometer. Water temperature (^{o}C) was measured 2 cm below the surface (Jung, 2002; Wood and Williams, 2013). Environmental variables including the pH, conductivity, and dissolved oxygen, were also recorded at the upper, middle, and lower point of each reach per sampling event using an YSI 556

Multi-probe meter (Yellow Springs Instruments; Yellow Springs, Ohio). These stream habitat variables were standardized by measuring at approximately the same time of day (prior to 1100), under similar weather conditions, and within a few days to avoid temporal bias.

Salamander Sampling

Within each stream, a 10-m reach that included the mesohabitat of a run, riffle, and pool was intensively sampled with all cover objects being searched for salamanders. Immediately upstream of the 10-m reach a 40-m reach was less intensively sampled with one cover object of at least 65 mm searched at every meter point of the reach. Within this 50 m, salamander abundance sampling also consisted of a 1-m terrestrial component on both sides of the stream to quantify adult salamanders utilizing the immediate habitat surrounding the stream in which all rocks and cover objects of at least 65 mm length and width were searched within the 10-m reach and at each 1-m point in the upstream 40-m reach. These salamander sampling reaches were located within the larger 100-m stream reach. Sampling occurred under appropriate weather conditions; i.e. not during extreme cold, heavy precipitation events, or strong winds (Williams, 2003; Wood and Williams, 2013). Each reach was thoroughly searched not only in the thalweg of the stream, but the streams entirety including the banks of the stream. Monorail dip-nets (10.5" x 8", depth 6") were used to aid capture of adult and larval salamanders and scoop under cover objects sampled.

Captured individuals were placed in a container of stream water filled to the approximate depth of the stream with placed cover objects to limit stress and possible consumption by other salamanders captured. For each individual captured; the age class (larvae or adult), species identification, and whether the individual was captured within the stream or within the terrestrial sampling component was recorded.

Aquatic Insect Sampling

Aquatic insects were sampled (March 2014) with four replicate Surber samples (0.09 m^2, 600 μm mesh) randomly stratified along the 100-m stream reach. All Surber samples were collected within the thalweg of a riffle mesohabitat within the stream (Pond, 2000). Once the random points were selected, the Surber sampler was placed within the thalweg of the stream and the substrate and cover objects that fell within the Surber sampler were dislodged and removed, thus capturing aquatic insects in the mesh of the Surber sampler. Debris, such as leaves and larger stones, were inspected for aquatic insects before being removed from the sample. Collected aquatic insects were separated by site into polyethylene bags and preserved in 70% ethanol before being transported to the laboratory for identification to Family using keys in Aquatic Insects of North America (4^{th} Edition; R.W. Merritt, K.W. Cummins, and M.B. Berg).

Data Analyses

To evaluate the differences among sampled reference streams, the habitat and environmental variables were reduced to Principal Components (PC) via Principal Component Analysis (PCA) using SPSS 22 (IBM SPSS Statistics, 2013). This process was undertaken as the number of sites was relatively low (n=8) compared to the number of stream variables measured. Only variables with a communality greater than 0.60 within the principal components were interpreted (Stevens, 1986). Sorenson similarity coefficients were calculated, and a Mantel Test was conducted to determine if salamander or aquatic insect community similarities between streams was a result of geographic distance between streams in the statistical program PC-ORD Multivariate Analysis of Ecological Data (MjM Software, Version 6, 2011). Shannon-Wiener Diversity indices and measures of evenness were also calculated to compare salamander species diversity among stream reaches. Aquatic insect metrics calculated for each stream included the total family richness, EPT (Ephemeroptera, Plecoptera, and Trichoptera) family richness, modified % EPT abundance, % Ephemeroptera, modified Hilsenhoff Biotic Index (E.P.A RBP For Wadeable Streams and Rivers 2nd edition; Resh et al, 1996, using data from Hilsenhoff, 1988), Family Shannon-Wiener Diversity indices, and overall abundance (Pond et al., 2003).

To evaluate the association of stream salamanders and aquatic insects to measured environmental and habitat variables, the environmental and habitat principal components (PC) were used as explanatory variables in reverse stepwise regression for the response variables of the relative abundances and diversity of salamander species per site and the relative abundances, %EPT, and family richness of aquatic insects per site using SPSS 22 (IBM SPSS Statistics, 2013). Principal components (PC) were also used as explanatory variables in reverse stepwise regression for each salamander species to determine the habitat or environmental variables that best predicted each species abundance. Significance was considered at alpha = 0.05 for all statistical tests.

RESULTS

Habitat and Environmental Characteristics

Water chemistry and larger scale habitat variables, including water depth, stream width, and canopy closure were generally consistent between stream reaches, with most variation associated with microhabitat features corresponding to the percentage composition of stream cover types (Table 1). Factor reduction and Principal Component Analysis (PCA) produced three principal components (PC) that predicted 77.12 % of the variance in habitat and environmental variables. PC_1 explained 30.13% of the variability in habitat and environmental variables and was heavily influenced by stream attributes including dissolved oxygen, stream width and fine particulate organic matter (FPOM)(Table 2). PC_2 explained 27.96% of the variability in habitat variables and was strongly influenced by water chemistry factors including pH, water temperature, and specific conductance, as well as the habitat feature bedrock (Table 2). PC_3 explained 19.02% of the variability in the habitat variables and was influenced by habitat features including canopy closure, and cobble and gravel cover objects within the stream (Table 2).

Table 1. Habitat and water chemistry data for eight reference stream reaches sampled in southeastern Kentucky (March–June 2014). Data are mean + SE derived from three points/ stream reach/month (n=9). BE= Big Everidge (Lilley Cornett Woods), BB= Bad Branch (Bad Branch State Nature Preserve), UT BB= unnamed tributary to Bad Branch (Bad Branch State Nature Preserve), MF= Martin's Fork (Martin's Fork Wildlife Management Area), HF= High Fork (Blanton Forest State Nature Preserve), WC= Watts Creek (Blanton Forest State Nature Preserve), SR= Sugar Run (Cumberland Gap National Historic Park), UT SR= unnamed tributary to Sugar Run (Cumberland Gap National Historic Park).

Parameter	BE	BB	UT BB	MF	HF	WC	SR	UT SR
Mean Temp (^oC)	14.82±1.12	10.84±0.21	13.05±0.45	12.08±1.05	13.73±0.70	13.88±0.84	14.1±1.00	13.89±0.93
Max Temp (^oC)	19.29	11.98	14.55	15.42	15.63	15.88	17.02	16.81
pH	6.36±0.19	4.25±0.26	4.64±0.26	4.31±0.20	5.72±0.09	5.64±0.15	5.70±0.14	5.86±0.08
Dissolved Oxygen (mg/L)	12.26±0.59	11.82±0.59	13.11±0.35	12.50±0.86	13.25±0.29	13.04±0.32	12.78±1.00	12.62±1.06
Specific Conductance (us/cm)	44.77±9.56	20.00±2.97	14.89±2.28	29.11±6.53	19.90±4.20	20.67±1.47	18.56±0.36	26.89±1.41
% Canopy Closure	69.78±3.39	68±5.74	75.11±3.80	79.11±2.89	71.33±4.37	70±5.67	60±1.11	68.22±3.33
Water Depth (cm)	4.44±0.79	10.86±1.94	9.98±1.56	11.93±0.78	5.33±1.10	8.89±1.84	16.82±1.63	11.55±1.99
Stream Width (m)	1.33±0.20	1.52±0.15	2.62±0.21	1.76±0.25	2.23±0.25	2.04±0.29	2.87±0.18	2.62±0.20
% Cobble	37.78±9.23	49.44±11.04	44.44±5.06	38.33±7.69	53.89±7.61	35.56±6.11	50±7.03	58.89±4.36
% Bedrock	31.67±12.52	0±0	0±0	0±0	0±0	0±0	0±0	0±0
% Gravel	20±5.27	12.22±4.09	40±6.16	23.33±3.51	15.56±3.46	37.22±9.56	16.11±3.91	15.78±2.49
% Coarse Woody Debris	0±0	7.22±4.52	0±0	3.33±2.08	5.56±3.64	5.56±2.41	0±0	6.67±3.24
% Moss	0±0	8.33±6.29	0±0	0±0	0±0	0±0	0±0	0±0
% Coarse Particulate Organic matter	0±0	3.88±3.66	0±0	18.88±8.04	0±0	0±0	0±0	0±0
% Fine Particulate Oranic Matter	10.55±4.04	10.55±7.21	22.77±4.17	21.11±6.52	17.5±6.50	21.11±5.97	33.89±6.13	18.67±2.38

Table 2. Table of Principal Component factor loading scores, eigenvalues, and the percent variance explained by habitat and environmental variables measured in eight reference stream reaches in southeastern Kentucky (March– June 2014).

Habitat and Environmental PCA	Axis 1	Axis 2	Axis 3
Eigenvalue	4.22	3.91	2.66
% Variance explained	30.132	27.96	19.02
p.H.	0.318	**0.813**	-0.425
Dissolved Oxygen (mg/L)	**0.767**	0.17	0.372
Water Temperature (^{o}C)	0.501	**0.845**	-0.124
Specific Conductance (ms/cm)	-0.549	**0.711**	-0.08
% Canopy Closure	-0.394	-0.056	**0.783**
Water Depth(cm)	0.45	-0.598	-0.093
Stream Width(m)	**0.945**	-0.221	-0.076
% Cobble	0.33	-0.351	**-0.697**
%Bedrock	-0.437	**0.844**	-0.112
%Gravel	0.269	0.14	**0.792**
%Coarse Woody Debris	-0.292	-0.479	-0.33
%moss	-0.588	-0.579	-0.392
Fine Particulate Organic Matter (FPOM)	**0.858**	-0.19	0.184
Coarse Particulate Organic Matter (CPOM)	-0.434	-0.375	0.521

Salamander Communities

A total of 390 streamside salamanders of 7 species were captured (Table 3). Larval salamanders accounted for 235 of the individuals captured; the remaining 155 salamanders captured were adults. Abundances of sampled salamanders ranged from 79 individuals at UT Bad Branch (Bad Branch SNP) to just 24 individuals at Sugar Run (Cumberland Gap NHP) (Table 4). Nearly half of captured salamanders (47%) were Southern Two-lined Salamanders (*Eurycea cirrigera*). While *Desmognathus* species represented 43% of captures with the remaining 10% consisting of Northern Red (*Pseudotriton ruber*) and Kentucky Spring salamanders (*Gyrinophilus porphyriticus duryi*). A Mantel test revealed that Sorenson's similarity indices between streams was not related to the geographic distance between stream reaches ($p=0.197$, $r^2=0.062$). Shannon-Wiener diversity indices ranged from 0.827 at Sugar Run to 1.677 at Bad Branch (Bad Branch SNP), with evenness scores for all sites ranging from 0.537 at Watts Creek (Blanton Forest SNP) to 0.862 at Bad Branch (Table 4). A two-tailed Spearman correlation test revealed salamander abundance was significantly correlated with aquatic insect richness ($r= 0.755$, $p=0.031$). No significant correlation was found with salamander richness and aquatic insect richness ($r= 0.327$, $p=0.429$), abundance ($r= 0.401$, $p=0.325$), or % E.P.T. ($r=0.375$, $p=0.359$).

Table 3. Salamander species detected by study site, among eight sampled reference stream reaches in southeastern Kentucky (April–June 2014).

Scientific Name	Common Name	BE	BB	UT BB	MF	HF	WC	SR	UT SR
Eurycea cirrigera	S. two-lined salamander	X	X	X	X	X	X	X	X
Desmognathus monticola	Seal salamander	X	X	X	X	X	X	X	X
Desmognathus welteri	Black Mtn. Dusky salamander	X	X	X	X	X	X		X
Desmognathus ochrophaeus	Allegheny Mtn. salamander	X	X	X	X				
Desmognathus fuscus	N. Dusky salamander	X	X	X	X	X	X	X	X
Gyrinophilus porphyriticus porphyriticus	Kentucky Spring salamander	X	X	X	X	X	X		X
Pseudotriton ruber	Red salamander	X	X	X		X	X		

Table 4. Salamander metrics including abundance, Shannon-Wiener diversity index, and evenness by study site, among eight sampled reference stream reaches in southeastern Kentucky (April–June 2014).

Metric	BE	BB	UT BB	MF	HF	WC	SR	UT SR
Abundance	65	43	80	47	35	53	24	44
Species Shannon H’	1.40	1.68	1.45	1.39	1.32	0.96	0.83	1.16
Evenness	0.72	0.86	0.75	0.78	0.74	0.54	0.75	0.72

Salamander abundance was significantly explained by PC_2 (water temp r= 0.845, bedrock r= 0.844, and pH r= 0.813, and specific conductance r= 0.711; Wald’s χ^2= 5.41, p=0.020) and PC_3 (gravel r= 0.792, canopy closure r= 0.783, and cobble r=-0.697; Wald’s

χ^2= 14.10, p<0.000). Reverse stepwise regression modeling did not reveal any significant predictors with salamander richness (Wald's χ^2=1.904, p=0.296). Principal components used in stepwise regression modeling significantly predicted for the abundance of nearly all salamander species, with the exception of Southern Two-lined Salamanders (*Eurycea cirrigera*). Species were generally predicted by stream chemistry factors including pH, specific conductance, and water temperature, and by habitat features including bedrock, cobble, and gravel cover objects within a stream (Table 5).

Table 5. Stepwise linear regression models predicting the abundance and presence of sampled salamander species from eight reference stream reaches (April–June 2014). Positive and negative signs refer to the observed significant (p<0.05) effect of the variable to each salamander species.

Species	Factor	Wald's χ^2	*P*
Eurycea cirrigera	Water Temperature (+) Bedrock (+) pH(+) Specific conductance (+)	1.513	0.219
Desmognathus monticola	Stream width (+) FPOM (+) D.O. (+)	9.99	0.002
	Water Temperature (+) Bedrock (+) pH(+) Specific conductance (+)	7.194	0.007

Table 5 (continued)

Species	Factor	Wald's χ^2	P
Desmognathus welteri	Stream width (+)	13.098	<0.001
	FPOM (+)		
	D.O. (+)		
	Water Temperature (+)	5.89	0.015
	Bedrock (+)		
	pH(+)		
	Specific conductance (+)		
	Gravel (+)	15.334	<0.001
	Canopy Closure (+)		
	Cobble (-)		
Desmognathus ochrophaeus	Water Temperature (+)	6.532	0.011
	Bedrock (+)		
	pH(+)		
	Specific conductance (+)		
	Gravel (+)	6.017	0.014
	Canopy Closure (+)		
	Cobble (-)		
Desmognathus fuscus	Water Temperature (+)	5.808	0.016
	Bedrock (+)		
	pH(+)		
	Specific conductance (+)		
Gyrinophilus porphyriticus porphyriticus	Water Temperature (+)	6.756	0.009
	Bedrock (+)		
	pH(+)		
	Specific conductance (+)		
	Gravel (+)	6.584	0.01
	Canopy Closure (+)		
	Cobble (-)		

The types of cover objects that predicted for salamanders, even within the *Desmognathus* genus, varied, although all *Desmognathus* species abundances were predicted for by PC2 (water temperature, bedrock, pH, and specific conductance). Northern Dusky (*Desmognathus fuscus*) and Seal (*Desmognathus monticola*) salamanders, were predicted by PC2 and in the case of the seal salamander by increasing dissolved oxygen, fine particulate organic matter, and stream width. While the more locally endemic Allegheny Mountain Dusky (*Desmognathus ochrophaeus*) and Black Mountain Dusky (*Desmognathus welteri)* salamanders were predicted by the decreased presence of cobble cover objects and increased canopy closure and gravel cover objects within a stream. The Kentucky Spring salamander (*Gyrinophilus porphyriticus porphyriticus*) abundance within a stream reach was predicted by PC2 as in the *Desmognathus genus*, as well as increasing canopy closure, gravel, and decreasing cobble cover objects.

Aquatic Insect Communities

A total of 1,142 aquatic insects representing 8 orders and 34 families were collected (Table 6). Family richness ranged from 21 (61.8% of the total families captured) at Big Everidge (Lilley Cornett Woods) to 13 families (38.2% of the total families captured) at Bad Branch (Bad Branch State Nature Preserve). Family richness within the Ephemeroptera, Plecoptera, and Trichoptera orders varied from 13 families at UT Sugar Run (Cumberland Gap National Historic Park) to 6 families at Sugar Run (Cumberland Gap NHP) and the overall % E.P.T. captured was 78.02%. The overall percent Ephemeroptera captured was 13.5%, with streams ranging from 0% at Bad

Branch to 22.3% at Big Everidge. Abundance values ranged widely from 381 at Big Everidge to 36 at Sugar Run (Table 7). A Mantel test revealed that Sorenson's similarity indices between streams was not related to the geographic distance between stream reaches ($p=0.192$, $r^2=0.064$). Modified Hilsenhoff biotic index levels and family Shannon diversity indices among sites ranged from 3.67–2.03 and 1.71–2.47, respectively (Table 7).

Table 6. Aquatic insect families detected and there abundance by study site for eight sampled reference stream reaches (March, 2014)

Family Name	BE	BB	UT BB	MF	HF	WC	SR	UT SR
Heptageniidae	11	0	0	0	3	3	5	31
Baetidae	3	0	0	0	0	1	0	0
Leuctridae	90	62	1	41	2	0	0	0
Hydropsychidae	50	3	42	9	38	13	7	12
Uenoidae	62	2	26	8	5	5	3	14
Elmidae	9	1	1	2	0	25	0	2
Ephemerellidae	71	0	0	1	14	0	1	2
Psychomyiidae	19	13	0	11	0	0	0	17
Psephenidae	13	0	2	0	10	3	5	7
Cordulagastridae	3	5	4	0	0	0	0	0
Corydalidae	2	0	0	0	0	0	0	0
Perlodidae	7	0	1	10	5	8	0	19
Nemouridae	5	0	17	0	1	0	7	2
Limniphilidae	1	0	0	0	0	1	0	0
Gomphidae	1	0	2	0	0	0	0	0
Aeshnidae	0	0	0	0	0	1	0	0
Philopotamidae	0	1	1	10	1	5	0	0

Table 6 (continued)

Family Name	BE	BB	UT BB	MF	HF	WC	SR	UT SR
Chloroperlidae	0	0	1	0	6	5	2	0
Perlidae	0	0	0	0	0	1	0	0
Tabanidae	0	0	0	0	1	3	0	0
Isonychiidae	0	0	0	0	1	1	0	0
Tipullidae	15	2	0	8	1	2	0	25
Ameletidae	0	0	0	4	0	0	0	2
Hydroptilidae	0	1	6	0	0	0	0	0
Glossosomatidae	0	0	0	0	0	0	2	4
Pteronarcyidae	0	0	0	0	0	0	0	4
Goeridae	0	0	0	0	0	0	0	2
Molanidae	0	0	0	0	0	0	0	1
Chironomidae	4	19	2	6	5	7	1	3
Simuliidae	2	8	1	14	0	0	2	10
Taeniopterygidae	0	5	2	14	0	0	0	1
Capniidae	3	0	1	2	0	0	0	0
Athericidae	1	2	0	0	0	0	1	1
Peltoperlidae	9	0	2	11	1	0	0	1

Table 7. Calculated aquatic insect family metric values for sampled aquatic insects from eight reference stream reaches across southeastern Kentucky (March, 2014).

Metric	BE	BB	UT BB	MF	HF	WC	SR	UT SR
Total Family Richness	21	13	17	15	15	16	11	20
EPT Family Richness	12	8	11	11	11	10	6	13
% EPT abundance	86.87	69.35	83.83	80.13	81.91	51.19	75	70
% Ephemeroptera	22.31	0	0	3.31	19.15	5.96	16.67	21.88
*Modified Hilsenhoff Biotic index(mHBI)	2.05	2.03	3.26	2.31	3.19	3.67	3.17	3.18
Family Shannon H'	2.28	1.71	1.92	2.38	2.01	2.29	2.17	2.47
Abundance	381	124	112	151	94	84	36	160

*Tolerance values used in the modified Hilsenhoff biotic index were developed for application in the E.P.A. Rapid Bioassessment Protocols For Use in Streams and Wadeable Rivers: 2nd edition (Bode et al, 1996; Hauer & Lamberti, 1996; Hilsenhoff, 1988; Plafkin et al, 1989).

Reverse stepwise regression models did not reveal any significant predictors with % EPT (Wald's χ^2= 0.857, p=0.355) or aquatic insect richness (Wald's χ^2= 2.152, p=0.142). Aquatic insect abundance was significantly explained by PC_1 (stream width r= 0.945, FPOM r= 0.858, and dissolved oxygen r=0.767; Wald's χ^2= 105.03, p<0.001), and by PC_2 (water temperature 0.845, bedrock r=0.844, pH r= 0.813, and specific conductance r= 0.711; Wald's χ^2= 183.85, p<0.001). Aquatic insect family richness models were not significant, however, a two-tailed Spearman correlation test revealed aquatic insect family richness was significantly correlated with aquatic insect abundance (r=0.790, p=0.014).

DISCUSSION

Trophic position, competition, and habitat availability drive the interactions and the presence of taxa within a stream ecosystem (Torgersen et al., 1999; Doi and Katano, 2008; Yeiser and Richter, 2015). Aquatic insects serve as the major prey base for central Appalachian headwater streams and specifically salamander species, the dominant predator within headwater streams. This study found evidence that these taxa responded similarly to environmental conditions as similar habitat and environmental variables predicted for the abundances of these two taxa within sampled headwater streams. A correlation was also found with salamander abundance and aquatic insect richness. The selection process of these streams as reference streams was supported by the habitat, water chemistry, and community data gathered. High diversity, evenness among sites, and abundances of both taxa support that these sites as possessing healthy communities and provides support for the importance of natural areas and the habitat they provide for central Appalachian streams. By understanding the factors that determine abundance and presence of these two taxa within central Appalachian headwater streams we can better understand ecosystem processes within these systems (Reice, 1991; Petranka, 1998; Davic and Welsh, 2004; Pond, 2010).

The percentages of E.P.T., family richness, and other important bioassessment metrics for aquatic insects was comparable to other studies conducted in reference streams in the area (Pond, 2000). Abundance and richness of salamander species also followed this trend (Muncy et al., 2014). This study supports that these taxa are important bioindicators of habitat quality within stream ecosystems (Pond et al., 2008, Welsh and Hodgson, 2013), as high abundances and diversity were observed in reference quality streams within the region. Factors including low conductivity (<250 us/cm) (Merriam et al., 2011) and high dissolved oxygen values likely contributed to the robust communities of salamanders and aquatic insects observed. Past studies have found high levels of conductivity and low dissolved oxygen values can negatively impact species in these communities due to reduced survivorship, physical abnormalities, and a reduced presence of oxygen for respiration (Pond et al., 2008; Merriam et al., 2011).

Another likely important factor leading to the community metrics observed for these taxa was the general lack of silt and sedimentation among cover objects. Increases in stream silt and sedimentation is often a byproduct of stream catchment disturbance, and has been found to be detrimental to aquatic communities because it can cause a lack of interstitial spaces among smaller cover objects, including gravel and cobble (Schwinghamer, 1981; Braccia and Voshell, 2007; Descloux et al., 2014). Increased sedimentation within aquatic systems can also act to disrupt the functioning of the gill surfaces of aquatic organisms (Soucek et al., 2000). Through the preservation and creation of natural areas, disturbance and therefore increased sedimentation can be limited and prevented in these important aquatic systems.

A Mantel test revealed differences among study sites were not a result of geographic distance, and were therefore likely a result of differences in stream habitat and water chemistry. Much of the variation seen between streams was observed at the microhabitat scale, with less variation occurring at large scale habitat features, largely accounted for in the site selection process. In general, habitat variables, such as water movement, substratum, and water chemistry, are important descriptors of community composition for stream organisms (Johnson et al. 2004; Doi and Katano, 2007). This study supports these previous studies because salamander and aquatic insect communities seemed to be generally driven by the types of cover available within the stream and water chemistry parameters.

As with sampled aquatic insect and salamander abundance, predictive models for each salamander species was also strongly determined by the stream bed morphology, available cover, and water chemistry variables. Variation was determined, however, between which types of cover predicted for each salamander species. No predictive model was created for southern two-lined salamanders, but this may be as this species is recognized as a generalist and found at high abundances both within heavily altered and relatively unaltered stream catchments (Weir et al., 2014). These results are supported by other studies, where microhabitat features differentially predicted the presence and abundance of stream salamander species (Yeiser & Richter, 2015).

Strong associations to microhabitat features in observed communities indicate that our predictive models are effective predictors of the presence and abundance of salamander and aquatic insect communities, but not richness across large landscapes, including southeastern Kentucky. The lack of predictive models for the richness of these two taxa in this study may be due to the limited variation and high richness observed across sites. By understanding the features that predict for stream salamander and aquatic insect species, we can hope to conserve appropriate habitat and therefore these important taxa across Appalachian aquatic ecosystems. In order to better understand the changes and threats posed to salamander and aquatic insect communities via anthropogenic change, it is important to determine natural variation in community composition and abundance across reference natural areas, to highlight the diversity and ecosystem functions that may be lost due to environmental disturbance.

This study highlights the importance of the creation and maintenance of natural areas, as they can serve as islands of suitable habitat, especially within a heavily disturbed landscape. By maintaining and preserving healthy forest stands within this region, it not only protects those forested systems, but also acts as a buffer of undisturbed habitat to preserve watersheds and protect aquatic biodiversity. Consideration must also be taken in the creation of new natural areas that promote connectivity to existing natural areas and the communities they support as human land use continues to spread.

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CHAPTER 2: EXAMINING THE IMPACTS OF VALLEY FILLS IN STREAM ECOSYSTEMS ON AMPHIBIAN AND AQUATIC INSECT COMMUNITIES IN SOUTHEASTERN KENTUCKY.

INTRODUCTION

Anthropogenic disturbance is one of the major contributors to declines in worldwide biodiversity (Dodd and Smith, 2003; Weyrauch and Grubb, 2003; Merriam et al., 2011). In the Appalachian region of the U.S., surface coal mining is one of the dominant drivers of human land-use change (Bernhardt and Palmer, 2011; Wood and Williams, 2013). Surface mining often converts large areas of what was once primarily mature hardwood forest into a modified landscape of reclaimed grasslands and shrubs of non-native species with fragmented pockets of forest (Brenner, 1985; Wickham et al., 2007). Mountaintop removal mining is a relatively recent approach to surface mining that converts Appalachian ridges and mountaintops to flattened plateaus via explosives and heavy machinery. This process results in large amounts of overburden that is deposited into valleys adjacent to mining sites, thus creating what is known as valley fills (Bernhardt and Palmer, 2011; Wood and Williams, 2013). These valley fills can be hundreds of hectares in size and permanently bury ephemeral, intermittent, and perennial streams; as of 2011 it’s estimated that over 2,000 km of headwater streams have been buried due to valley fill operations (Bernhardt and Palmer, 2011).

In addition to the direct loss of headwater stream habitat, environmental impacts also affect streams below valley fills (Palmer et al., 2010; Wood and Williams, 2013). For example, water quality of streams below valley fills is impaired by high concentrations of metals, including magnesium, manganese, mercury, potassium, and selenium and elevated levels of specific conductance, sulfate concentrations, pH, and sedimentation (Pond et al., 2008; Merriam et al., 2011). Decreased water quality of stream ecosystems due to valley fill operations has been linked to declines in Appalachian stream biodiversity in multiple taxa including macroinvertebrates and salamanders (Pond et al., 2008; Merriam et al., 2011; Wood and Williams, 2013) in an ecoregion recognized as a global hotspot for biodiversity and endemism (Wickham et al., 2007; Bernhardt and Palmer, 2011).

In Appalachian ecosystems, salamanders perform many key ecological functions (Marcot and Vander Hayden, 2001; Davic and Welsh, 2004). In terms of abundance or biomass, salamanders are often the dominant vertebrate predators in aquatic and terrestrial systems (Burton and Likens, 1975; Davic and Welsh, 2004). Because of their relatively high abundance and complex life cycles, salamanders are important links between invertebrate and vertebrate communities and serve a critical role in transferring energy between terrestrial and aquatic ecosystems (Petranka, 1998; Davic and Welsh, 2004; Hopkins, 2007). Therefore, loss of salamander populations from headwater streams of Appalachia has ecosystem-wide consequences (Petranka, 1998; Davic and Welsh, 2004; Welsh and Hodgson, 2013).

Aquatic insects serve many functions in aquatic ecosystems, including regulation of nutrients via breakdown of organic material by shredder and decomposer feeding guilds, and impact levels of decomposition, productivity, and translocation of material within stream systems (Wallace and Webster, 1996). Therefore, because of the high diversity and abundance of aquatic insects in stream systems, changes in feeding guild structure can significantly alter how nutrients are regulated within a stream (Reice, 1991; Pond, 2010). Aquatic insect diversity is particularly high in headwater streams (Stout and Wallace, 2005; Clarke et al., 2008), and they serve as a major prey base in aquatic ecosystem food webs (Pond et al., 2008), and specifically represent the major source of food for aquatic amphibians in stream ecosystems of Appalachia (Jackson et al., 2007).

When assessing stream health, organisms are often used as bioindicators because of their utility as indicators of physical or chemical characteristics and reflection of cumulative impacts over time (Tebo, 1955; Davis et al., 1996; Hutchens et al., 2004). Stream salamanders and aquatic insects are two taxa that have been identified in multiple studies as bioindicators (Wallace et al., 1988; Lowe and Bolger, 2002; Pond et al., 2008; Welsh and Ollivier, 1998). They are useful bioindicators of headwater streams because of their high abundance, ease of sampling, sensitivity to changes in the quality of habitat, and propensity for bioaccumulation of contaminants from the environment (Welsh and Ollivier, 1998; Pond et al., 2008; Welsh and Hodgson, 2013). However, we are unaware of research focused on evaluating stream salamanders and aquatic insects as bioindicators simultaneously despite their connected role in aquatic food webs, key ecological functions, and role as bioindicators (Resh et al., 1988; Davic and Welsh, 2004; Welsh and Hodgson, 2013).

Due to the complex nature of many contaminants in aquatic ecosystems, they often bioaccumulate in organisms and can be passed into higher trophic levels of the food web (Goodyear and McNeill, 1999; Walter et al., 2008). In aquatic ecosystems throughout the U.S., selenium has become a primary element of concern because of its ability to readily bioaccumulate in organisms (Hamilton, 2004; Orr et al., 2005; Bergeron et al., 2010a). However, there has been a lack of research in how selenium bioaccumulates in stream salamanders and aquatic insects relative to other taxa despite their key ecological functions and role as bioindicators of habitat quality (Wallace et al., 1988; Davic and Welsh, 2004; Pond et al., 2008; Bergeron et al., 2010a). The appearance of selenium in aquatic ecosystems has been associated with mining activity (Conley et al., 2009; Wood and Williams, 2013). However, previous research has primarily focused on selenium in stream ecosystems derived from coal-fired plants (Unrine et al., 2007), with little research examining selenium concentrations in streams impacted by valley fills.

The goal of this research was to determine the impacts of valley fills to aquatic ecosystems of Appalachia by comparing impacted streams with reference streams in terms of habitat quality, stream salamander and aquatic insect communities, and selenium concentration in organisms and the environment. It was predicted that valley fills within streams negatively impact stream quality and salamander and aquatic insect communities, and therefore, that a less abundant and diverse salamander and aquatic insect community would be observed at the valley-fill streams and that water and stream habitat quality would be lower than observed reference stream quality. Additionally, it was predicted that the levels of selenium bioaccumulation within sampled organisms and the environment would be higher in valley-fill streams.

METHODS

Study Area

Sampling occurred in reference streams (RS) with no mining history and streams directly impacted by valley fills (VFS). RS sites consisted of mature, forested first-order headwater streams considered to be some of the best quality headwater streams in the region based on discussions with personnel from the Kentucky Division of Water, Kentucky State Nature Preserves, and Kentucky Natural Lands Trust. Stream catchment size varied from 2.46-3.52 sq. miles in RS sites and 1.69-4.23 sq. miles at VFS sites. The forest stands were at least 70 years old, including old-growth forest, and the headwaters of the streams and sampled stream reaches were within national and state protected area boundaries. These protected areas are located north of Pine Mountain and on the north and south side of Black and Cumberland mountains in Bell, Harlan, and Letcher counties (Fig. 2). VFS sites consisted of first-order streams, with sampled stream reaches located within 500 meters of the valley fill site. Sampled VFS were located in second-growth forest of varying maturity, geographically located within 15 km of RS sites (Fig. 2) Although VFS sites had lower forest canopy closure, all sites were forested and during site selection care was taken to attempt to control for the canopy closure around a site.

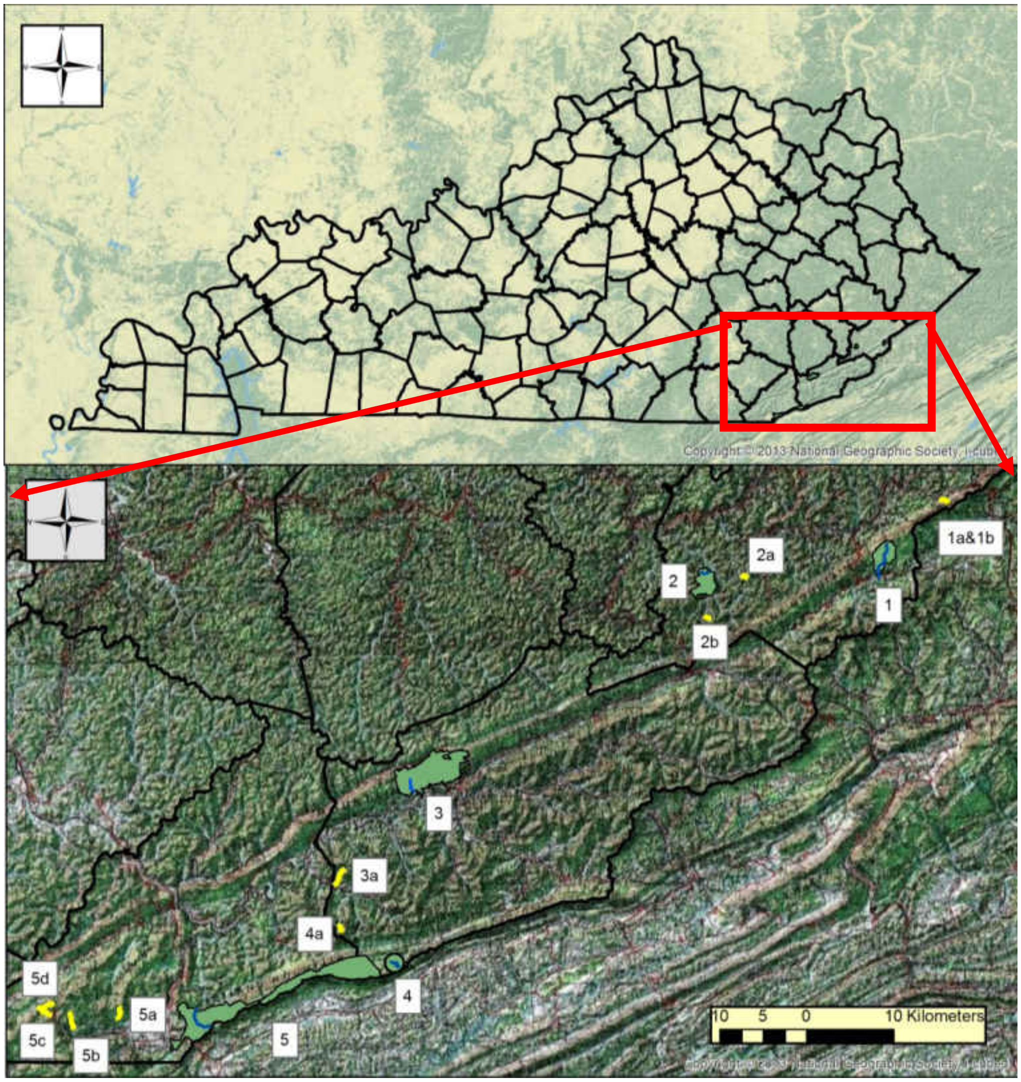

Figure 2. Study sites in southeastern Kentucky sampled (March–June 2015). Moving from east to west, reference sites include Bad Branch State Nature Preserves (1), Lilley Cornett Woods (2), Blanton Forest State Nature Preserve (3), Martin's Fork State Natural Area (4), and Cumberland Gap National Historic Park (5). One stream was sampled per natural area. Labels, including numbers and letters, refer to valley fill sites, with the number corresponding to its paired reference site.

Study Design

Sampling occurred in five reference first-order streams and ten first-order streams impacted by valley fills. Each sampled reference stream was paired with two valley-fill streams that were located within 15 km's in March–June 2015. Within each stream, a

100-m transect was positioned 20 meters above the confluence of each headwater stream to a larger stream, to decrease the likelihood of the stream drying and standardize placement of the stream reach. Habitat sampling occurred four times in 1-month intervals in the spring (March–June) of 2015, while collection of salamanders occurred three times in 1-month intervals (April–June 2015), with aquatic insects (March 2015) and water sample (May 2015) collection consisting of a single sampling event. All sites were sampled within four days of one another per sampling event and at least 48 hours since the last precipitation event. The order of sampling between study streams was randomized to avoid temporal bias.

Habitat Sampling

The dominant mesohabitat, cover types, canopy closure, water depth (cm), stream width (m), and water temperature (^{o}C) were measured at three sampling points corresponding to the upper, middle, and lower points of each reach (i.e. at 0, 50, and 100 m) per sampling event. At each point, the proportion of dominant mesohabitat types (run, riffle, and pool) and cover types (silt, sand, gravel, pebble, cobble, boulder, muck, and detritus) were estimated based on a view looking directly down upon the stream (Jung, 2002; Wood and Williams, 2013). The amount of canopy closure was visually estimated using a spherical densiometer. Water temperature (^{o}C) was measured 2 cm below the surface (Jung, 2002; Wood and Williams, 2013). Environmental variables including the pH, conductivity, and dissolved oxygen, were also recorded at the upper, middle, and lower (i.e. 0-, 50-, and 100-m) point of each reach per sampling event using an YSI 556 Multi-probe meter (Yellow Springs Instruments; Yellow Springs, Ohio). These stream habitat variables were standardized by measuring at approximately the same time of day (prior to 1100), under similar weather conditions, and within a few days to avoid temporal bias. Collection of water samples for metal analysis consisted of collecting 10 ml of stream water approximately 2 cm below the surface from three points (i.e. 0, 50, and 100 m) of each sampling reach. Water samples were placed immediately on ice and chilled no longer than 48 hours before acidifying samples with the addition of 100 µL of concentrated HNO_3.

Salamander Sampling

Within each stream, a 10-m reach that included the mesohabitat of a run, riffle, and pool was intensively sampled with all cover objects being searched for salamanders. Immediately upstream of the 10-m reach a 40-m reach was less intensively sampled with one cover object of at least 65 mm searched at every meter point of the reach. Within this 50 m, salamander abundance sampling also consisted of a 1-m terrestrial component on both sides of the stream to quantify adult salamanders utilizing the immediate habitat surrounding the stream in which all rocks and cover objects of at least 65 mm length and width were searched within the 10-m reach and at each 1-m point in the upstream 40-m reach. These salamander sampling reaches were located within the larger 100-m stream reach. Sampling occurred under appropriate weather conditions; i.e. not during extreme cold, heavy precipitation events, or strong winds (Williams, 2003; Wood and Williams, 2013). Each reach was thoroughly searched not only in the thalweg of the stream, but the streams entirety including the banks of the stream. Monorail dipnets (10.5” x 8”, depth 6”) were used to aid capture of adult and larval salamanders and scoop under cover objects sampled.

Captured individuals were placed in a container of stream water filled to the approximate depth of the stream with placed cover objects to limit stress and possible consumption by other salamanders captured. For each individual captured, the age class (larvae or adult), species identification, and whether the individual was captured within the stream or within the terrestrial sampling component was recorded.

Salamander Metal Analysis

In July of 2014 a pilot study was conducted to test the viability of a non-destructive tail-clipping technique for metal analysis. A small subset of salamanders captured at a valley-fill stream was sacrificed in order to separately quantify the bioaccumulation of metal concentrations in body tissue and clipped tails (Bergeron et al., 2010b). A two-tailed Spearman correlation test revealed a strong correlation ($r=0.840$, $p<0.001$) between the metal concentrations in body tissue and tail tissue. Therefore, only tail clips were taken from sampled individuals in the primary sampling season (April–June 2015) to reduce mortality of sampled organisms.

To standardize sampling, the tail of salamanders were removed 20 mm above the tip using a sterile blade and weighed, following rinsing with stream water and body condition measurements (Bergeron et al., 2010b). An attempt was made to collect 15 tail clips from each stream site to be used in metal analysis. However, due to the low abundances found at several valley-fill sites less than 15 tail clips were collected. A total of 165 tail clips were taken and used in selenium analysis. The clipped tail was kept cool at 4 C^o before being lyophilized and the dried weight was recorded (Bank et al., 2005). Clipped tails were then digested in 750 µL of trace metal grade nitric acid HNO3 in fluoropolymer digestion vessels using a microwave digestion system (MARS-5, CEM) according to U.S. EPA method 3052 (U.S. E.P.A., 1996). After digestion, the samples were brought to a final volume of 15ml with >18 MΩ deionized water. Analytical method blanks and the standard reference material TORT-2 lobster heptopancreas (National Research Council of Canada, Ottawa, ON) were included in each digestion batch. Selenium analysis was performed on diluted samples through Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) according to U.S. EPA method 6020a (U.S. E.P.A., 1998).

Aquatic Insect Sampling

Aquatic insects were sampled (March 2015) with four replicate Surber samples (0.09 m^2, 600 µm mesh) randomly stratified along the 100-m stream reach. All Surber samples were collected within the thalweg of a riffle mesohabitat within the stream (Pond, 2000). Once the random points were selected, the Surber sampler was placed within the thalweg of the stream and the substrate and cover objects that fell within the Surber sampler were dislodged and removed, thus capturing aquatic insects in the mesh of the Surber sampler. Debris, such as leaves and larger stones, were inspected for aquatic insects before being removed from the sample. Collected aquatic insects were separated by site into polyethylene bags and preserved in 70% ethanol before being transported to the laboratory for identification to Family using keys in Aquatic insects of North America (4th edition; R.W. Merritt, K.W. Cummins, and M.B. Berg).

Data Analyses

Habitat and Environmental Characteristics

Non-parametric Mann-Whitney U-tests were performed for evaluating the differences between treatments in habitat and environmental variables because data violated the assumptions of normality. Analysis of differences in selenium concentrations in water between RS and VFS sites were performed via two-sample t-tests. Tests for differences between treatments were conducted using SPSS 22 (IBM SPSS Statistics, 2013).

Salamander Communities

To evaluate the differences between sampled RS and VFS sites two-sample t-tests were performed between salamander richness, abundance, abundance by species, and selenium concentrations. Shannon-Wiener Diversity indices and measures of evenness were also calculated to compare salamander species diversity among stream treatments via two-sample t-tests. Tests for differences between treatments were conducted in the statistical program SPSS 22 (IBM SPSS Statistics, 2013).

To account for imperfect detection of salamanders, estimates were developed for salamander abundances and occupancy for VFS and RS, through the binomial mixture model developed by Royle (2004), with alterations by Price et al. (2012). This model estimates abundance and individual detection rate, while incorporating site-level and survey covariates and provides estimates of the uncertainty associated with each parameter. This procedure was not completed for sampled aquatic insects as the field protocol described by Royle (2004) consists of replicate counts, whereas aquatic insects were sampled via 4 spatially explicit points only one time.

In conducting these models salamander count data were separated by species and life stage (i.e. larval or adult), as well as by treatment type (VFS or RS). We assumed that the detectability of salamanders might also differ among sites and among visits due to date since last precipitation and Julian date, so these factors were included in the model as covariates.

To estimate population parameters for each stage and species, WinBUGS Version 1.4 in batch mode with data handling in R (Spiegelhalter et al., 2003) (version 2.10) (add-in library R2Win- BUGS) was used. Posterior summaries for each parameter were based on 100,000 Markov chain Monte Carlo iterations with a 10,000 sample burn-in and a thinning rate of 3. The mean and standard deviation of the model coefficients were calculated, along with the 2.5 and 97.5 percentiles of the distribution, representing 95% Bayesian credible intervals. Abundance estimates were derived using the log transformation presented by Price et al. (2012), with RS represented by 0 with VFS represented by 1.

The total abundances of salamanders sampled were determined as the total number captured for each stream transect and was also compared between treatments through nonmetric multidimensional scaling (NMS) in the statistical program R 3.1.2 (Ihaka and Gentleman, 1996) Vegan package (2014) using the Bray-Curtis similarity coefficient (Bray and Curtis, 1957). Sites were grouped and labeled by treatment as valley fill (VF) and reference stream (RS) sites. Stress values below 20% were considered satisfactory.

Aquatic Insect Communities

To evaluate the differences between sampled RS and VFS sites two-sample t-tests were performed between aquatic insect abundance, family richness, percent E.P.T. (Ephemeroptera, Plecoptera, and Trichoptera orders), percent Ephemeroptera, E.P.T. family richness, and modified Hilsenhoff Biotic Index values (E.P.A RBP For Wadeable Streams and Rivers 2nd edition; Resh et al, 1996, using data from Hilsenhoff, 1988). Shannon-Wiener Diversity indices and measures of evenness were also calculated to compare aquatic insect family diversity among stream treatments via two-sample t-tests. Tests for differences between treatments were conducted in the statistical program SPSS 22 (IBM SPSS Statistics, 2013).

The total relative abundances of aquatic insects sampled were determined as the total number captured for each stream transect and compared between treatments through nonmetric multidimensional scaling (NMS) in the statistical program R 3.1.2 (Ihaka and Gentleman, 1996) Vegan package (2014) using the Bray-Curtis similarity coefficient (Bray and Curtis, 1957). Sites were grouped and labeled by treatment as valley fill (VF) and reference stream (RS) sites. Stress values below 20% were considered satisfactory.

RESULTS

Habitat and Environmental Characteristics

Mann-Whitney U-tests determined that over half of measured habitat and environmental variables significantly differed between treatments, with factors equating to stream size (stream width, stream depth, and dissolved oxygen) being similar between treatments (Table 8). Two-sample T-tests revealed that the presence of selenium was significantly higher in water samples in VFS (mean=0.741 µg/L, n=30, SE=0.190) than in sampled RS (mean=0.011 µg/L, n=15, SE=0.015) (t=-2.697, df=43; p=0.010).

Table 8. Habitat and environmental variables measured by treatment in sampled valley-fill (n=10) and reference (n=5) streams in southeastern Kentucky (March–June 2015).

Parameter	Valley-fill streams (Mean $\pm$ SE, n=10)	Reference streams (Mean $\pm$ SE, n=5)	*p*-value
Dissolved Oxygen (mg/L)	11.133 $\pm$ 0.171	11.399 $\pm$ 0.323	0.548
pH	7.947 $\pm$ 0.018	5.542 $\pm$ 0.197	<0.001
Water Temperature (C)	14.347 $\pm$ 0.501	12.082 $\pm$ 0.735	0.003
Specific Conductance (µs/cm)	719.100 $\pm$ 0.039	34.200 $\pm$ 0.003	<0.001
Canopy Closure %	67.083 $\pm$ 1.712	80.466 $\pm$ 1.541	<0.001
Water Depth (cm)	13.645 $\pm$ 0.632	11.735 $\pm$ 0.639	0.168
Stream Width (m)	2.021 $\pm$ 0.082	2.046 $\pm$ 0.110	0.538
Boulder %	17.125 $\pm$ 1.829	11.501 $\pm$ 1.993	0.121
Cobble %	19.501 $\pm$ 1.633	48.891 $\pm$ 2.656	<0.001
Gravel %	22.901 $\pm$ 1.466	19.625 $\pm$ 1.880	0.267
Silt %	39.208 $\pm$ 1.766	3.000 $\pm$ 1.077	<0.001
Coarse Woody Debris (CWD) %	0.875 $\pm$ 0.494	3.300 $\pm$ 0.867	<0.001
Coarse Particulate Organic Matter (CPOM) %	0.208 $\pm$ 0.149	2.583 $\pm$ 0.792	<0.001

Salamander Communities

In this study we captured 529 individual salamanders of eight species, with captures in RS (n=335) higher than in VFS (n=194). We captured seven species in both RS and VFS, however, no more than four species of salamander were captured at any VFS site. Species richness was significantly higher is RS (mean ± SE = 6.2 ± 0.20) than VFS (mean ± SE = 2.9 ± 0.41) (t=-5.482, df =13; p<0.001). The four species of *Desmognathus* were the most frequent captures and accounted for 52.8% of captures in RS (n=177), and 69.6% of captures in VFS (n=135). No captures were recorded for *Desmognathus* *welteri* in VFS. While the Southern two-lined salamander (*Eurycea cirrigera*) represented 30.1% in RS and 27.3% in VFS, with the remaining 16% in RS and 2% in VFS consisting of Kentucky Spring (*Gyrinophilus porphyriticus*) and Northern Red salamanders (*Pseudotriton ruber*). A single capture of the Long-tailed salamander (*Eurycea longicauda*) was recorded at a VFS.

Salamander abundance was also significantly lower in VFS (mean ± SE = 19.4 ± 3.63) than sampled RS (mean ± SE = 67.0 ± 7.74) ($t=-6.337$, $df=13$; $p<0.001$) and most of the species sampled were found in significantly lower abundances in VFS than sampled RS (Table 9). Shannon-Wiener diversity indices were significantly higher in RS (mean ± SE = 1.47 ± 0.10) than in sampled VFS (mean ± SE = 0.69 ± 0.13) ($t=-4.233$, $df=13$; $p=0.001$), and evenness was higher in RS (mean ± SE = 0.81 ± 0.05) than VFS (mean ± SE = 0.57 ± 0.11), but not significantly different between treatments ($t=-1.448$, $df=13$; $p=0.171$).

Table 9. Salamander species mean abundance with standard error detected by treatment in sampled valley-fill (n=10) and reference streams (n=5) in southeastern Kentucky (April–June 2015). *Pseudotriton ruber* and *Eurycea longicauda* were not examined via two-sample t-test due to their limited abundance, and only the total amount captured is given.

Sampled Species	Valley-fill streams (Mean ± SE, n=10)	Reference stream (Mean ± SE, n=5)	p-value
Desmognathus fuscus	1.3±0.63	4.4±2.29	0.11
Desmognathus monticola	4.4±2.15	14.8±5.20	0.043
Desmognathus ochrophaeus	7.6±2.65	5.2±1.74	0.559
Desmognathus welteri	0±0	8.6±3.23	0.002
Eurycea cirrigera	5.3±2.27	20.2±4.57	0.006
Gyrinophilus porphyriticus	0.2±0.2	8.6±2.15	p<0.001
Pseudotriton ruber	3	15	
Eurycea longicauda	1	0	
Total captured	194	335	
Average abundance/stream	19.4	67	

Detection probabilities varied by salamander species and life stage among covariates with days since last precipitation and Julian date having positive, negative or no effects (i.e. 95% CI overlaps with zero)(Table 10). Some consistent relationships did occur including the relationship between salamander detection and Julian date was always positive (i.e. higher detection with increasing date since January 1st) because 95% credible intervals did not contain zero (Table 10). Detection of salamander species was also influenced by days since last precipitation; however, this relationship was not consistent among species. For example a negative relationship was found with *D. monticola* and *E. cirrigera* adults and larvae, but a positive relationship was found with *D. ochrophaeus* adults (Table 10).

Table 10. Detection parameters, including mean and 95% credible interval, and detection probabilities for adult and larval salamanders in both VFS and RS sites. Variation in detection was modeled with the covariates days since last precipitation (D.S.P) and Julian date. Several species were removed due to low abundances including E. longicauda and P. ruber. The abundances of adult and larval G. porphyriticus were combined, as well as total salamander estimates.

Sampled species	parameter	Adult	Larvae
Desmognathus fuscus	D.S.P.	-0.71(-1.99,0.40)	-0.47(-20.05,19.46)
	Julian date	0.56(-0.48,1.63)	-0.09(-19.52,19.29)
	Detection Probability	0.11	0.98
Desmognathus monticola	D.S.P.	-0.73(-1.36,-0.09)	-1.56(-3.07,-0.30)
	Julian date	-0.43(-0.94,0.04)	-0.12(-1.07,0.79)
	Detection Probability	0.18	0.04
Desmognathus ochrophaeus	D.S.P.	1.15(0.77,1.57)	0.49(-1.61,2.47)
	Julian date	0.06(-0.27,0.40)	2.69(0.10,6.31)
	Detection Probability	0.05	0.07
Desmognathus welteri	D.S.P.	1.66(-6.43,11.41)	-0.59(-20.38,19.46)
	Julian date	11.59(1.49,25.76)	0.01(-19.38,19.42)
	Detection Probability	1.03	0.97
Eurycea cirrigera	D.S.P.	-1.38(-2.46,-0.38)	-1.18(-1.68,-0.72)
	Julian date	-0.40(-0.97,0.17)	0.03(-0.30,0.35)
	Detection Probability	0.09	0.04
Gyrinophilus porphyriticus	D.S.P.	0.38(-1.51,2.09)	
	Julian date	0.89(-0.74,0.82)	
	Detection Probability	0.09	
Total salamanders	D.S.P.	-0.10(-0.38,0.19)	
	Julian date	-0.11(-0.35,0.12)	
	Detection Probability	0.79	

Considerable variation in species and stage specific estimates of salamander abundance was determined between treatments, with most estimates including large credible intervals. In general, however, abundance estimates were higher for RS than

VFS, with the exception of *D. ochrophaeus* adults and larvae and *D. monticola* larvae. Effects due to treatment (i.e. positive effect included positive values with 95% credible intervals that did not overlap with zero, negative effect included negative values with 95% credible intervals non-overlapping with zero, and no effect which include 95% credible intervals that overlapped with zero) were observed for a few species, with no effects determined for several species collected in low abundances (Figure 3). Negative effects due to mining were observed with *D. monticola* and *E. cirrigera* adults, as well as with *E. cirrigera* larvae and total salamanders (Figure 3). Positive effects were found with mining in abundance estimates for *D. ochrophaeus* (Figure 3).

Analysis of the accumulation of selenium in the tissue of salamander tails revealed that levels were significantly higher in sampled VFS (mean ± SE = 2.76 ± 0.25 mg/kg dry mass, n=92) than in RS (mean ± SE = 1.59 ± 0.091mg/kg dry mass, n=73) (t=-4.014, df=163; p<0.001).

NMS produced a 2-dimensional solution with a satisfactory stress value of 13.11% (Figure 4). A Shepherd plot of the data revealed a strong linear fit (R^2=0.983) of the model. Species loading scores were generally higher on NMS axis 2, especially some of the species found at lower abundances, including Black Mountain and northern dusky salamanders, and Kentucky Spring and Northern Red salamanders (Figure 4). The higher loading of species scores on NMS axis 2 was consistent with the loading values of RS sites (Figure 4). There was a lack of separation between VFS and RS in ordination space which indicates shifts in community structure were not as strongly determined by mining activity in a few of the VFS sites (Figure 4).

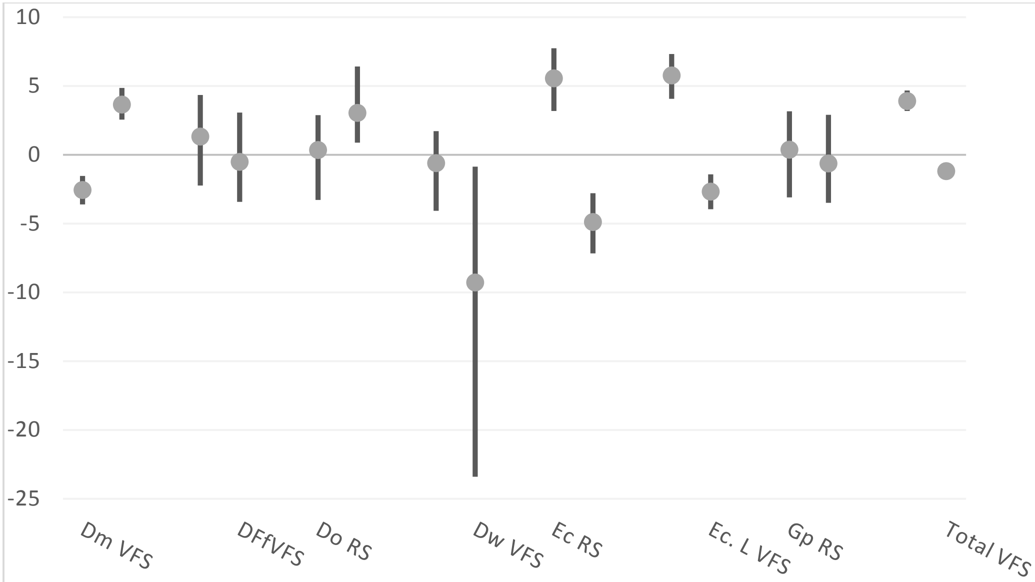

Figure 3. Estimates of the effect of mining on abundances of adult *Desmognathus monticola* (Dm), *Desomgnathus fuscus* (Df), *Desmognathus ochrophaeus* (Do), *Desmognathus welteri* (Dw*), Eurycea cirrigera* (Ec), *Gyrinophilus porphyriticus* (Gp), and larval *Eurycea cirrigera* (Ec L) and total salamanders detected in valley fill (VFS, n=10) and reference streams (RS, n=5). Error bars indicate 95% credible intervals. Species and/or stages with parameter estimates (including 95% credible intervals) below zero indicate a decline due to valley fills in streams. Larval salamanders of most species were not included due to detection in low abundances.

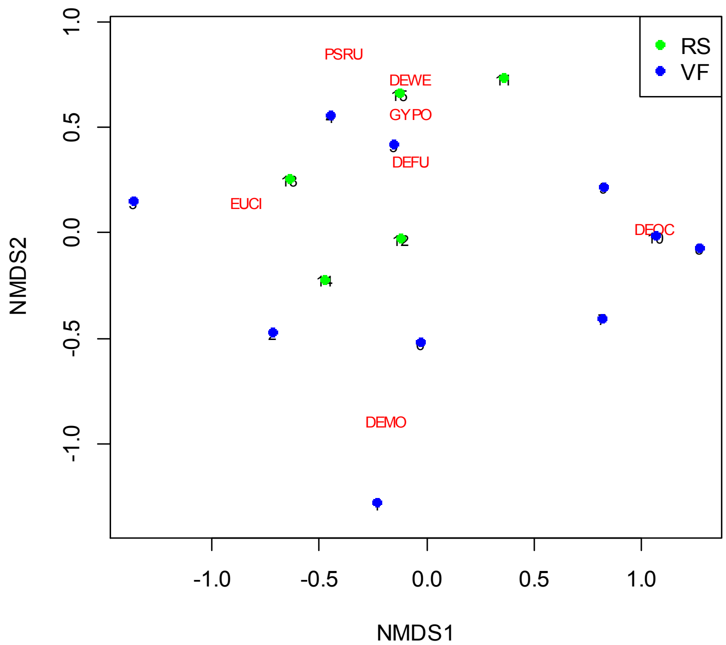

Figure 4. Nonmetric multidimensional scaling ordination for salamander species at sampled reference streams (RS, n=5) and valley-fill streams (VF, n=10) in southeastern Kentucky (April–June 2015). EUCI = Southern two-lined salamander (*Eurycea cirrigera)*; DEMO = Seal salamander (*Desmognathus monticola*); DEWE = Black Mountain salamander (*Desmognathus welteri*); DEOC = Allegheny Mountain dusky salamander (*Desmognathus ochrophaeus*); DEFU = Northern Dusky Salamander (*Desmognathus fuscus*); Kentucky Spring salamander (*Gyrinophilus porphyriticus*); PSRU= Northern Red salamander (*Pseudotriton ruber*).

Aquatic Insect Communities

A total of 1,034 aquatic insects representing 8 orders and 37 families were collected, and more families were captured in RS (n =32) than VFS (n = 25). Aquatic insect abundance was found to be significantly lower in VFS (total captures = 447; mean ± SE = 44.7 ± 5.9) than sampled RS (total captures = 587; mean ± SE =117.4 ± 38.0) (t=-2.682, df=13; p=0.019) (Table 11). Family richness was also found to be significantly lower in VFS (mean ± SE = 9.6 ± 0.64) than sampled RS (mean ± SE = 16.2 ± 2.0) (t=-

4.048, df=13; p=0.001) (Table 11). The EPT family richness was also found to be significantly lower in VFS (mean ± SE = 7± 0.557) than sampled RS (mean ± SE = 11.8 ± 1.496) (t=6.651, df=13; p=0.002) (Table 11).

The percentage of E.P.T. was found to be higher in RS (mean ± SE = 84.3 ± 3.0) than VFS (mean + SE = 78.8 ± 4.8) (Table 11), but not significantly higher (t=-0.763, df=13;p=0.459). The percentage of Ephemeroptera was not significantly different between VFS (mean ± SE =19.15 ± 5.493) and RS (mean ± SE =12.42 ± 5.185) (t= 0.787, df=13; p=0.451) sites (Table 11). Although tolerance values were lower in RS (mean ± SE = 2.71 ± 0.19) than VFS (mean ± SE = 3.08 ± 0.17) (Table 11), they were not significantly different (t=1.369, df=13; p=0.194). Aquatic insect Shannon-Wiener diversity indices were significantly higher in RS (mean ± SE = 2.22 ± 0.083) than in sampled VFS (mean ± SE = 1.78 ± 0.098) (t=-2.919, df=13; p=0.012). Aquatic insect evenness was found to be only slightly higher in RS (mean ± SE =0.802 ± 0.009) than VFS (mean ± SE = 0.792 ± 0.027), but not significantly different (t=-0.248, df=13; p=0.808) between treatments, as with sampled salamanders.

Table 11. Calculated aquatic insect family metrics in valley-fill (n=10) and reference (n=5) streams in southeastern Kentucky (March 2015).

Macroinvertebrate metric	Valley-fill streams (Mean ± SE)	Reference streams (Mean ± SE)	*p*-value
Family Richness	9.6 ± 0.636	16.2 ± 1.985	0.001
Total Abundance	44.8 ± 5.944	117.8 ± 38.023	0.019
Percent EPT Abundance	78.8 ± 4.763	84.3 ± 3.033	0.459
mHBI Values	3.1 ± 0.168	2.7 ± 0.186	0.194
Shannon-Wiener Diversity index	1.781 ± 0.098	2.225 ± 0.083	0.012
Evenness	0.792 ± 0.027	0.802 ± 0.009	0.808
% Ephemeroptera	19.15 ± 5.49	12.42 + 5.185	0.451
EPT Family Richness	7 ± 0.557	11.8 ± 1.496	0.002

NMS produced a 2-dimensional solution with a satisfactory stress value of 16.27% (Figure 5). A Shepherd plot of the data revealed a strong linear fit (R^2=0.974) to the created NMS model. Family loading scores ranged widely with NMS axis 1 and 2, with no clear single axis explaining the aquatic insect family abundances (Figure 5). However, VFS sites were separated considerably from RS sites on NMS axis 1 in ordination space, which indicates that shifts in community structure were associated with mining activity (Figure 5). The higher loading of sensitive families on NMS axis 1 including the stoneflies Capniidae and Leuctridae, and the caddisfly Glossosomatidae was consistent with the loading values of RS sites (Figure 5).

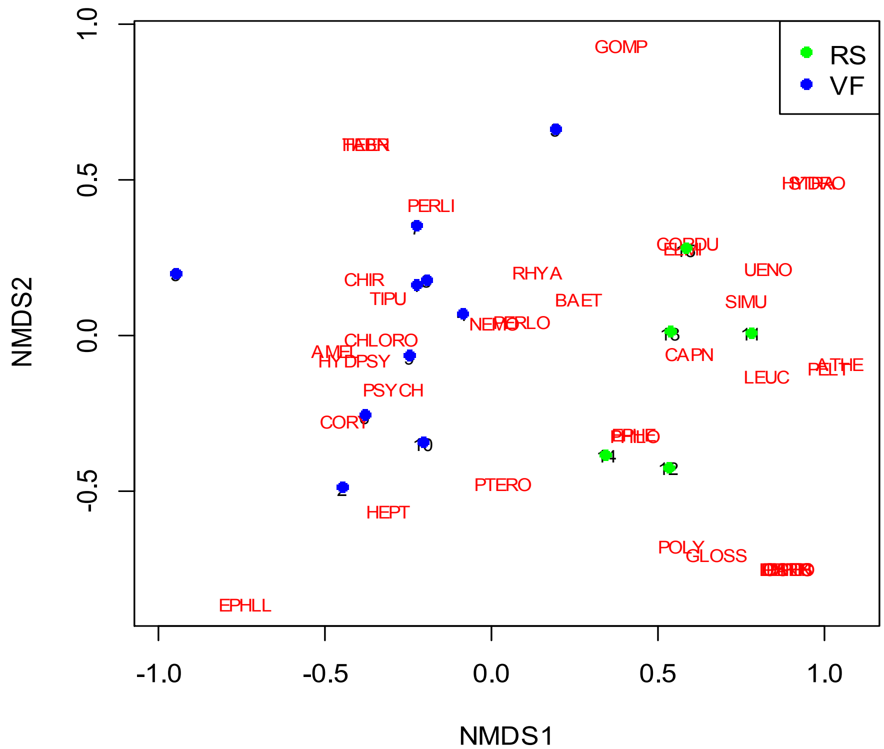

Figure 5. Nonmetric multidimensional scaling ordination for aquatic insect families at sampled reference streams (RS, n=5) and valley-fill streams (VF, n=10)in southeastern Kentucky (March 2015). Macroinvertebrate family codes: AMEL=Ameletidae; BAET=Baetidae; TAEN=Taeniopterygiidae; NEMO=Nemouridae; CHIR=Chironomidae; HEPT=Heptageniidae; PERLO=Perlodidae; HYDPSY=Hydropsychiidae; TIPU=Tipullidae; PERLI=Perlidae; HEBR=Hebridae; RHYA=Rhyacophillidae; PSYCH=Psychomyiidae; CORY=Corydalidae; LEUC=Leuctridae; CORDU=Cordulagastridae; CAPN=Capniidae, CHLORO=Chloroperlidae; EPHE=Ephemeridae; EPHLL=Ephemerellidae; ELMI=Elmidae, GOMP=Gomphidae; UENO=Uenoidae; PTERO=Pteronarcyidae; SIMU=Simuliidae; PHILO=Philopotamidae; PELT=Peltoperlidae; ATHE=Athericidae; PHRY=Phryganeidae; GLOSS=Glossosomatidae; PSEP=Psephenidae; DYTIS=Dytiscidae; CAEN= Caenidae; LEPTO=Leptophlebiidae, POLY=Polycentropidae; HYDRO=Hydroptilidae; STRA=Stratiomyiidae.

DISCUSSION

Headwater streams are often buried due to valley fill processes (Bernhardt and Palmer, 2011). In addition to the outright loss of headwater streams, ecological impacts including altered habitat and environmental characteristics have been documented (Palmer et al., 2010; Bernhardt and Palmer, 2011; Wood and Williams, 2013). We found that sampled VFS possess altered habitat and environmental characteristics and less rich and diverse stream salamander and aquatic insect communities. In sampled RS, salamander and aquatic insect abundance, diversity, and Shannon-Wiener diversity indices were significantly higher than in sampled VFS. The percentage of E.P.T. typically found at high abundances in Central Appalachian streams and used in bioassessment (Pond et al., 2008; Bourne and Richter in review), were higher at RS than VFS. Based on tolerance values for aquatic insects, and the NMS ordination for salamander species and aquatic insect families, generally more tolerant communities were found at VFS compared with higher diversity, abundance, and sensitivity of taxa at RS sites.

The reduced abundance and diversity of salamander and aquatic insects may be due to a host of interacting habitat and environmental variables observed at VFS. Features related to stream size such as width, depth, and dissolved oxygen were consistent among RS and VFS, suggesting that the paired approach of sample sites was effective in controlling for stream size and catchment area. However, many features relating to the types of cover and environmental features were significantly different between stream types. For example, silt cover was 13 times greater in VFS than in RS. Silt cover negatively affects small stream organisms as it fills crucial interstitial habitat spaces between and under cover objects used by salamander species and aquatic insect prey (Redmond, 1980; Lowe and Bulger, 2002; Wood and Williams, 2013; Muncy et al., 2014). Silt cover likely contributed to decreased abundance and diversity of salamander and aquatic insects observed in our study.

VFS sites had a greater presence of very large cover objects (boulders) and very small ones (gravel) compared to medium sized cover objects (cobble), which provided less available habitat for these taxa (Table 1). Additionally, large cover objects can increase habitat availability for predators of these two taxa, Martin et al., 2012 found salamander species were significantly more abundant in medium sized cover and predators including large crayfish species were significantly more abundant in large cover objects. Therefore, low cobble availability for refugia and increased boulders contribute to explaining the reduced communities observed at VFS. Lower forest canopy closure was also found in VFS sites compared with RS, and other studies have found that streams with reduced forest cover have lower salamander abundance and occupancy (Price et al., 2011, 2012; Muncy et al., 2014). Although VFS sites had lower forest canopy closure, all sites were forested and during site selection care was taken to attempt to control for the canopy closure around a site. Reduced canopy closure around a stream site can also lead to increased water temperatures and sedimentation, which may negatively impact taxonomic richness and exclude the occupancy of sensitive taxa (Wood & Armitage, 1997; Braccia and Voshell, 2006).

Water quality of VFS study sites was impaired with elevated levels of conductivity, 21 times higher than in RS. Several studies on the effects of mountaintop removal mining and valley fills on water chemistry reflect our findings with Pond et al., 2008 finding levels 16.5 times higher. High levels of conductivity have been identified as a contributing factor in the decreased abundance and distribution of salamander species because of reduced survivorship and physical abnormalities, as well as a decrease in aquatic insect prey items (Karraker et al., 2008; Wood and Wiliams, 2013; Muncy et al., 2014). Decreases in aquatic insect populations in VFS have been documented due to water chemistry and specifically the presence of high conductivity (Pond et al., 2008; Pond, 2010), which most likely contributed to the decreased abundance, richness, and presence of sensitive families at our VFS sites.

A confounding result was observed at VFS and RS sites, with no difference determined between the percentages of Ephemeroptera between stream types. Pond et al. 2008 found a significantly higher percentage of Ephemeroptera at RS sites, as well as a nearly complete loss of Ephemeroptera downstream of highly impacted VF sites. Observed Ephemeroptera percentage was low among both RS and VFS sites alike and may be caused by sampling within small watershed headwater streams. Streams sampled had relatively small watershed sizes and the intermittent nature of some of these streams may have caused the low abundances of Ephemeroptera observed. Different sampling methodologies were also employed in the Pond et al. 2008 study which used larger kick-nets possibly leading to the differences in richness observed, as well as a greater range of specific conductance values were detected including several sites with greater than 2,000 µs/cm. As this study only identified stream insects to family this may also explain the percentage of observed Ephemeroptera, as there are varying levels of tolerance among insect families and perhaps more tolerant genera were observed among VFS sites.

Incorporating estimates of detection and covariates that might affect the detection of an organism is important when studying secretive species such as salamanders (Royle, 2004; Price et al., 2012). Estimated salamander abundances and detection probabilities varied widely between species and life stage. For a few of our salamander species, days since last precipitation and Julian date had an effect on abundance, but generally these covariates did not have a large effect on our estimates. The main effect observed in our abundance estimates was between treatments. The sparseness and lower abundances of some species (*Desmognathus* larvae, *D. fuscus*, *D. welteri,* and *G. porphyriticus*) resulted in large credible intervals in our estimates and general lack of observed effect. Although large credible intervals were found around some of our species estimates, estimates were higher for each species in RS than VFS and when species were combined, increasing our sample size, estimates were significantly higher in RS than VFS sites. Effects between treatments were also found with species captured in higher abundances (*E. cirrigera* adults and larvae, *D. monticola* adults). Lack of significance with some of our sampled species was due to capturing them at lower abundances, decreasing our statistical power. Two-sample t-tests between the abundances observed between treatments revealed that all sampled salamanders except for D. fuscus and D. ochrophaeus were found in significantly lower abundances in VFS sites.

Due to the complex nature of many contaminants in aquatic ecosystems, they often bioaccumulate in organisms and can be passed into higher trophic levels of the food web (Goodyear and McNeill, 1999; Walter et al., 2008). In aquatic ecosystems throughout the U.S., selenium has become a primary element of concern because of its ability to readily bioaccumulate in organisms and cause reduced function, survivorship, and reproductive success (Hamilton, 2004; Orr et al., 2005; Bergeron et al., 2010a). We found an increased presence of selenium in water and tissue samples collected from VFS. The appearance of selenium in aquatic ecosystems has been associated with mining activity (Conley et al., 2009; Wood and Williams, 2013), however, the presence of selenium within our collected water samples was lower than values detected in other valley fill studies (0.74 μg/L vs. 8.6 μg/L) (Wood and Williams, 2013).

The reduced presence of selenium we detected in water samples may have been due to factors including the age since valley fill construction, the local geology of southeastern Kentucky study sites compared to studies in the coal fields of West Virginia, or the increased flashiness of valley-fill streams due to reduced habitat complexity (Bernhardt and Palmer, 2011). These factors may also have influenced the levels found in tissue, as a reduced presence in water would lead to a reduced availability to accumulate within an organism occupying that habitat. Selenium averages for VFS sites was lower than criterion levels set by the USEPA (2004) for fish tissue (7.91 mg/kg), however, several sampled salamanders tissue levels exceeded 11 mg/kg in VFS sites.

Results from this study provide further evidence of depressed aquatic insect and salamander communities, and impaired habitat and environmental quality in streams impacted by valley fills. Previous studies have evaluated salamander (Wood and Williams, 2013; Muncy et al., 2014) or aquatic insect communities (Pond et al., 2008; Pond 2010), or environmental characteristics (Metts et al., 2012), but to date we know of no studies that have evaluated these parameters simultaneously in these systems. By conducting research on these taxa simultaneously, this study provides valuable information about the habitat and environmental factors that act to exclude or lead to decreased abundance and richness of salamander and aquatic insect communities in Appalachian headwater streams. Through determining that similar factors in theses streams reduced both salamander and aquatic insect communities we can make conclusions about these taxonomic groups in studies that have only evaluated salamander or aquatic insect communities (Pond et al., 2008; Muncy et al., 2014). By approaching the issue of the health of Appalachian streams through multiple research questions, this study provides a broader understanding of the effects of valley fills on the health of salamander and aquatic insect communities and highlights the reduction in valley-fill stream quality and function.

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