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LIMITED IMPACTS OF ACID RUNOFF FROM PYRITE-BEARING ROCK FORMATIONS ON STREAM SALAMANDERS IN MIDDLE TENNESSEE HEADWATER STREAMS

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Abstract.— In addition to the myriad anthropogenic disturbances that impact biota and conservation efforts, acidification of aquatic habitats is an important threat to aquatic biodiversity. In this study, we examined the effects of acid rock drainage (ARD) on stream salamander assemblages in streams associated with rock cuts and pyrite-bearing Chattanooga and Fentress Shale formations in Middle Tennessee. We selected two streams monitored by the United States Geological Survey for changes in water quality and used a paired study design to evaluate the impacts of ARD by monitoring sites above and below the ARD disturbance. We surveyed a single transect and two quadrats (each surveyed once) in each paired stream reach at both sites and captured 158 larval and adult stream salamanders of 6 species. Salamander counts were similar for adult and larval salamanders between ARD-impacted and unimpacted stream reaches. Biodiversity measures (species richness, diversity, and evenness) did not differ between ARD-impacted and unimpacted stream reaches. Similarly, adult and larval counts for the Spotted Dusky Salamander (*Desmognathus conanti*) and Southern Two-lined Salamander (*Eurycea cirrigera*) did not differ between reaches. In terms of species composition, adult and larval *E. cirrigera* captures represented 37.9% and 94.2% of captures in ARD-impacted reaches versus 6.7% and 84.2% of captures in unimpacted reaches. We did not detect significant differences in water quality measurements (pH, temperature, and dissolved oxygen) between ARD-impacted and unimpacted stream reaches. We attribute the lack of stream disturbance effects to mitigation measures (limestone rock abatements) that were implemented above ARD-impacted stream reaches after road cut disturbance. Collectively, our pilot study provides an initial examination of the impacts of ARD on stream salamander assemblages in Tennessee, and suggests that impacts at these two sites are limited. However, a broader scale and replicated field study is necessary before larger conclusions can be established.

Key Words.—acid rock drainage; *Desmognathus*; disturbance; *Eurycea*; lotic; Plethodontidae; riparian

The disappearance of amphibians is occurring globally at a rate of nearly 200 times the background extinction rate primarily through anthropogenic means, including commercial use, disease, climate change, pollution, introduction of invasive species, and habitat destruction (Blaustein et al. 1994; Stuart et al. 2004; Beebee et al. 2005; Cushman 2006; McCallum 2007). Given the large-scale decline in amphibian biodiversity, it is important to evaluate the impacts of human disturbances on potentially vulnerable species and create management plans to mitigate the

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effects of human disturbance on population, biodiversity, and ecosystem health.

Amphibians contribute directly to ecosystem function through a variety of ecosystem services. In particular, salamanders contribute directly to carbon sequestration (Wyman 1998; Best et al. 2014), predation of detritivores (Brodman and Dorton 2006), as prey in trophic food webs (Davic and Welsh 2004), through movement of nutrients between aquatic and terrestrial environments (Regester et al. 2008), and as biological indicators of community fitness (Welsh and Droege 2001).

The southeastern United States exhibits high salamander diversity and provides an excellent opportunity to utilize these amphibians as biological indicators of ecosystem condition (Micacchion 2002; Bailey et al. 2004; Welsh and Droege 2007). Many of these salamander species occur in low-order, headwater streams that originate from ground-water seeps and represent the top predators in these ecosystems (Davic and Welsh 2004). Understanding abundance and richness of stream salamander assemblages can provide valuable data relating to water quality, habitat suitability, and impacts of ecological disturbance. These species are also sensitive to stream side disturbances that impair riparian buffers or water quality (Barrett and Price 2014), especially when these disturbances impact the underlying geology of riparian zones.

Pyrite and similar minerals that contain sulfur and trace metals occur in rock formations associated with low-order streams throughout the eastern and southeastern United States (Cook et al. 1994; Daniels and Orndorff 2003; Anderson 2008). When pyrite is exposed to oxygen and water through disturbance of bedrock layers, the minerals decompose and the sulfur can react to form sulfuric acid, resulting in environmental contamination (Bacon and Maas 1979; Daniels and Orndorff 2003; Hammarstrom et al. 2004). When these pyrite-bearing formations are exposed in a road cut (e.g. an area where a section of rock or soils is cut or blasted away to make space for transportation infrastructure), there is potential for acid rock drainage (ARD hereafter) that can also contain elevated levels of heavy metals, which may have a negative impact on stream ecosystems (Bacon and Maas 1979). These compounds can be released and transported during rain events (Hammarstrom et al. 2004), and if the runoff is untreated, there may be unintended negative consequences on aquatic biodiversity and environmental conditions (Kucken et al. 1994; Schorr et al. 2013).

Anthropogenic disturbances, such as mining and road construction, can greatly influence headwater stream diversity through direct habitat impacts and changes in water chemistry (Bernhardt and Palmer 2011). Previous studies have shown that aquatic acidification can negatively impact aquatic invertebrates (Niyogi et al. 2002) and vertebrates, including both fishes and amphibians (Huckabee et al. 1975; Kucken et al. 1994; Schorr et al. 2013). Stream salamanders display species- and life-stage specific sensitivities to reduced pH, with mortality occurring below pH levels of 4.2, and larvae having greater sensitivity compared to adults (Green and Peloquin 2008). Further, the geographic distribution of stream salamander larvae (*Desmognathus spp.* in particular) is negatively impacted by landscape disturbance, most notably watersheds with reduced pH and increased turbidity and sedimentation (Gore 1983). Landscape disturbances such as mountaintop removal mining result in large reductions of available habitat, but also cause rapid reductions in stream pH and increases in specific conductivity and dissolved solids, which also negatively impact stream salamanders (Muncy et al. 2014; Price et al. 2016). Studies that have evaluated the impacts of ARD on stream salamanders has been limited; however, streams impacted by ARD have displayed decreases in pH and increases in metals and near elimination of macroinvertebrates, fish, and salamanders (Mathews et al 1981; Kucken et al. 1994) which illustrates that ARD disturbance can potentially impact stream biodiversity, including salamanders.

To this end, we aimed to evaluate the impacts of ARD disturbance on stream salamander assemblages in Tennessee headwater stream ecosystems. Our small-scale survey, which was limited to two sites, examined the potential impacts of ARD on stream salamanders at streams in Middle Tennessee in two different geologic shale formations. We predicted that impaired stream reaches would have lower diversity, richness, abundance and greater evenness of adult and larval salamanders compared to unimpacted stream reaches. We also predicted that larval counts for all salamander species would be impacted to a greater extent compared to adult salamanders due to the fully aquatic nature of this life stage and greater sensitivity of this life stage to reduced water quality parameters.

METHODS AND MATERIALS

Study Sites—. We selected study sites in Chattanooga and Fentress shale formations in Middle Tennessee that coincided with an on-going ARD project implemented by the United States Geological Survey (USGS). We targeted two sites in Middle Tennessee with direct drainage from ARD disturbance into neighboring low-order streams. The first site included a road cut through a geologic formation that contained Chattanooga Shale located in south Williamson County (840 site), which drains to the headwaters of Carter's Creek, which, in turn flows into the Mill Creek watershed (Figure 1 A – D; 35.814735, -86.972729). Road construction at the site began in 2011 as a continuation of Interstate 840 construction. The impacted portion of the stream was located ~ 100 m from the road cut. The second ARD site located in Fentress County (Fentress site) cuts through the Fentress Shale formation, and was a result of a road-cut made for Highway 127 constructed between 2008 and 2009. Runoff from this ARD disturbance runs off 430 m directly into a headwater stream of the Wolf Creek watershed (Figure 1 E – H; 36.493378, -84.963707). Specific conductance and pH measurements in water runoff samples at both ARD road cuts indicated that specific conductance was elevated (840 site: 926 – 2266 µS/cm; Fentress site: 1726 – 1870 µS/cm) and pH measurements were below neutral (840 site: 2.45 – 4.00; Fentress site: 5.25 – 6.82; Byl, unpublished data). Both sites were low-order (e.g., Order 1 and 2) headwater stream environments that were mitigated with limestone rock between the riparian zone of the study streams and ARD road disturbance.

Salamander Sampling—. We conducted field surveys (each site and stream reach surveyed once) during September and October of 2015 to evaluate biological impacts of ARD on stream salamander assemblages. We used a paired experimental design that included one stream reach located above the ARD disturbance (i.e., unimpacted) and a reach that was located downstream of the ARD disturbance (i.e., ARD-impacted) at each of the two sites. This design permitted a direct and relative measure of ARD disturbance on stream salamanders. We identified stream segments within each stream reach that contained habitat features (e.g., rock cover, small riffles, permanent water) conducive for the presence of stream salamanders as recommended in Jung (2002).

We used stream quadrat and transect surveys as described in Price et al. (2011) to evaluate the impacts of ARD on adult and larval stream salamanders. We delineated one 15 m x 3 m linear transect and two 1 m x 1 m quadrats that spanned both terrestrial and aquatic habitat in ARD-impacted and unimpacted stream reaches at both sites. We

FIG. 1. Summary photographs for two headwater streams impacted by acid rock drainage (ARD) disturbance in TN. Photographs A – D represent the 840 site (Williamson County, TN) as follows: A) highway 840 road cut with iron staining, B) close-up of pyrite-bearing rock layers, C) upstream (unimpacted) section of the 840 site, D) downstream (ARD-impacted) section of the 840 site. Photographs E – H represent the Fentress site (Fentress County, TN) as follows: E) highway 127 road cut, F) ARD runoff from the base of the highway 127 road cut, G) iron staining on tree roots and soil substrate where runoff from the highway 127 road cut enters the Fentress County stream, and H) upstream (unimpacted) section of the Fentress site.

surveyed for and captured adult and larval stream salamanders opportunistically by turning over cover objects, including rocks and logs within the transect and quadrat boundaries. We surveyed transects using non-destructive methods (i.e., cover objects were briefly lifted and returned), whereas we surveyed quadrats using destructive methods (i.e., all cover objects were completely removed from the quadrat grid and returned at the conclusion of the survey). We used these two survey methods to obtain estimates of both larval and adult salamander abundance. We identified each captured salamander to species and measured (snout-vent length [mm]) and weighed mass (g) using a digital scale.

Environmental Data—. We measured stream pH, water temperature, and dissolved oxygen at the downstream end of each ARD-impacted and unimpacted stream reach prior to implementation of stream surveys. We used an Oakton® EcoTestr™ pH2^{+} meter to monitor stream pH and a YSI® EcoSense® DO200 Series meter to monitor both water temperature (°C) and dissolved oxygen (ppm). We calibrated all instruments as specified by the manufacturer.

Data Analysis—. We determined total salamander counts and percent composition for species with > 10 captures between stream reaches above and below the ARD disturbance. We also determined richness, evenness, and Simpson's Diversity Index (heterogeneity) separately for the ARD-impacted and unimpacted stream reaches as defined in Krebs (1999). We used paired t-tests in RStudio v.1.1.414 to evaluate differences among species diversity measures (richness, evenness, and heterogeneity) and relative abundance for larval and adult stream salamanders. We considered relationships statistically-significant when p-values were ≤ 0.05.

RESULTS

Salamander Sampling—. We captured 158 salamanders of 6 species (Table 1). The Spotted Dusky Salamander (*Desmognathus conanti*) was the most commonly-captured adult salamander species (47 captures) and the Southern Two-lined Salamander (*Eurycea cirrigera*) was the most commonly-captured larval salamander species (85 captures; Table

TABLE 1. Total counts and percent composition of adult and larval stream salamanders in streams monitored for biological impacts of acid rock drainage.

Species	Species Count	% of Captures
Adult		
Desmognathus conanti (Spotted Dusky Salamander)	47	74.60%
Desmognathus welteri (Black Mountain Salamander)	1	1.60%
Eurycea cirrigera (Southern Two-lined Salamander)	12	19.00%
Eurycea lucifuga (Cave Salamander)	1	1.60%
Plethodon dorsalis (Northern Zig-zag Salamander)	2	3.20%
Total Adults	**63**	**100%**
Larvae		
Desmognathus conanti (Spotted Dusky Salamander)	5	5.30%
Eurycea cirrigera (Southern Two-lined Salamander)	85	89.40%
Gyrinophilus porphyriticus (Spring Salamander)	5	5.30%
Total Larvae	**95**	**100%**

1). The Black Mountain Salamander (*Desmognathus welteri*) and the Cave Salamander (*Eurycea lucifuga*) were both captured once at the Fentress County site and the 840 site, respectively (Table 1). We captured five larval Spring Salamanders (*Gyrinophilus porphyriticus*) at the Fentress County site (Table 1).

We did not detect impacts of ARD disturbance on adult salamander diversity measures: species richness (t = 0.00, df = 1, p = 1.00), diversity (t = –0.47, df = 1, p = 0.72), and evenness (t = 1.00, df = 1, p = 0.50); larval diversity measures: species richness (t = 1.00, df = 1, p = 0.50), diversity (t = –1.93, df = 1, p = 0.30), and evenness (t = –0.19, df = 1, p = 0.88); and total salamander diversity measures: species richness (t = 0.33, df = 1, p = 0.80), diversity (t = –2.32, df = 1, p = 0.26), and evenness (t = –0.30, df = 1, p = 0.82) between ARD-impacted and unimpacted stream reaches (Figure 1).

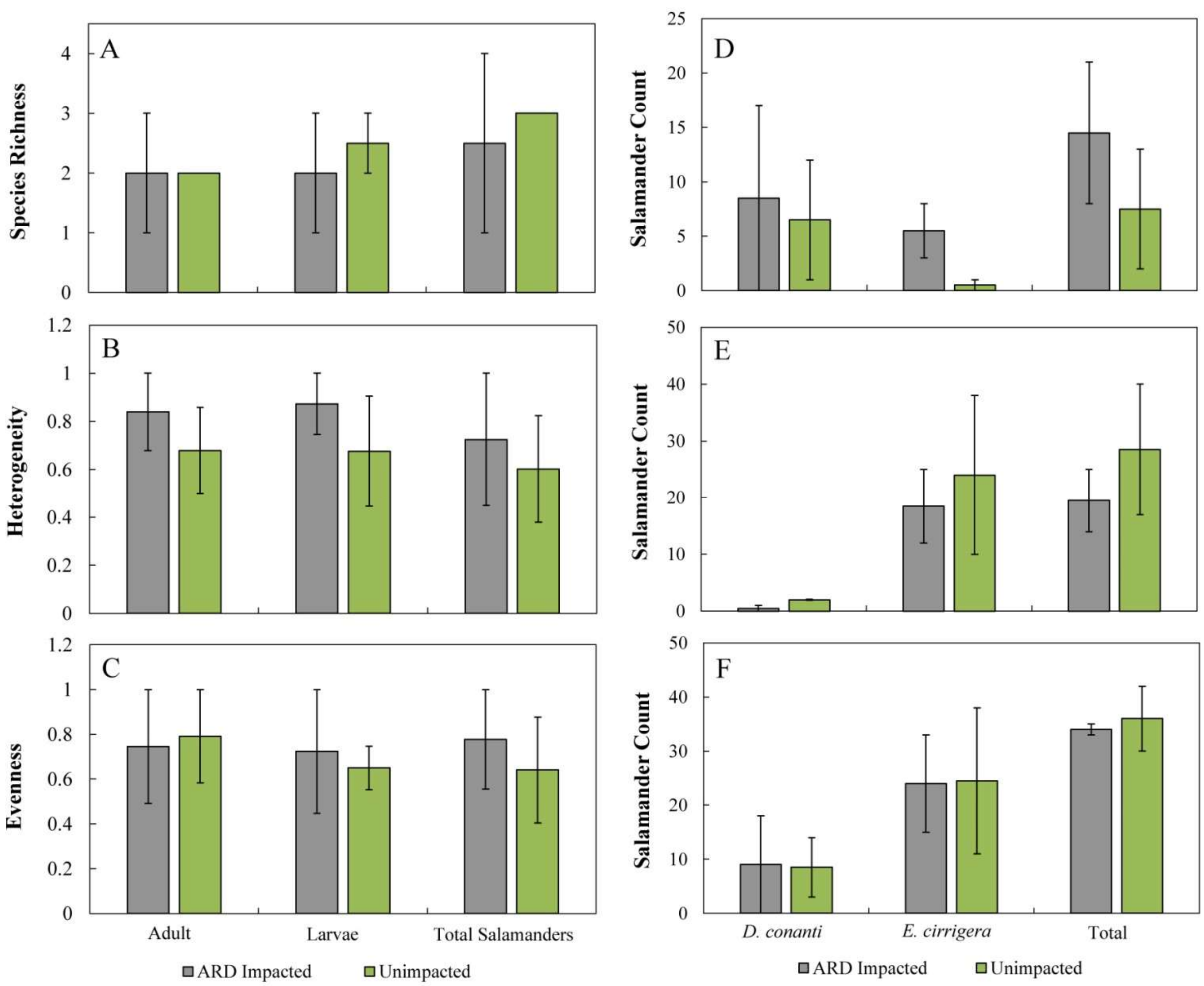

FIG. 2. Impacts of ARD disturbance on richness (A), heterogeneity (B), and evenness (C) for adult, larval, and total salamander counts. In addition, impacts of ARD disturbance on total counts for Spotted Dusky (*Desmognathus conanti*) salamanders, Southern Two-lined (*Eurycea cirrigera*) salamanders, and both species combined (Total) for adults (D), larvae (E), and total counts (F) of both larvae and adults. In all figures, gray bars represent ARD-impacted stream reaches and green bars represent unimpacted reaches.

Although total adult salamander counts were greater in ARD-impacted stream reaches compared to unimpacted reaches (Figure 2), this difference was not significant (t = –7.00, df = 1, p = 0.09; Figure 2). This trend was due to relative abundance of adult *E. cirrigera*, which although greater in ARD-impacted stream reaches (5.5 ± 2.5 captures) compared to unimpacted (0.5 ± 0.5 captures) stream reaches (Figure 2), was not statistically significant (t = –1.67, df = 1, p = 0.34). Larval counts averaged 28.5 ± 2.5 in unimpacted stream reaches and 19.5 ± 2.0 in ARD- impacted stream reaches, but were not statistically significant (t = 1.50, df = 1, p = 0.50). In addition, differences for larval *D. conanti* (t = 3.00, df = 1, p = 0.20) and *E. cirrigera* (t = 0.73, df = 1, p = 0.60) between ARD-impacted and unimpacted stream reaches were not statistically significant (Figure 2). We did not detect an effect of ARD disturbance on total salamander counts (i.e., larval and adult salamanders combined; t = 2.85, df = 1, p = 0.82) and found that percent composition was largely similar between ARD-impacted and unimpacted reaches (Figure 2). Anecdotally, we observed a trend of greater percent species composition of *E. cirrigera* in ARD-impacted stream reaches (adults: 37.9% of captures; larvae: 94.8% of captures) compared to unimpacted stream reaches (6.7% of captures; 84.2% of captures) across both sites (Figure 3).

Environmental Data—. We did not detect statistically-significant differences between ARD-impacted and unimpacted stream reaches for pH (t = 0.67, df = 1, p = 0.63; ARD-impacted: 7.45 ± 0.45, unimpacted: 7.85 ± 0.15), water temperature (t = 3.0, df = 1, p = 0.20; ARD-impacted: 12.0 ± 2.0 ᴼC, unimpacted: 12.5 ± 2.0 ᴼC), or dissolved oxygen (t = -2.084; df = 1, p = 0.28; ARD-impacted: 9.74 ± 0.28 ppm, unimpacted: 9.31 ± 0.31 ppm).

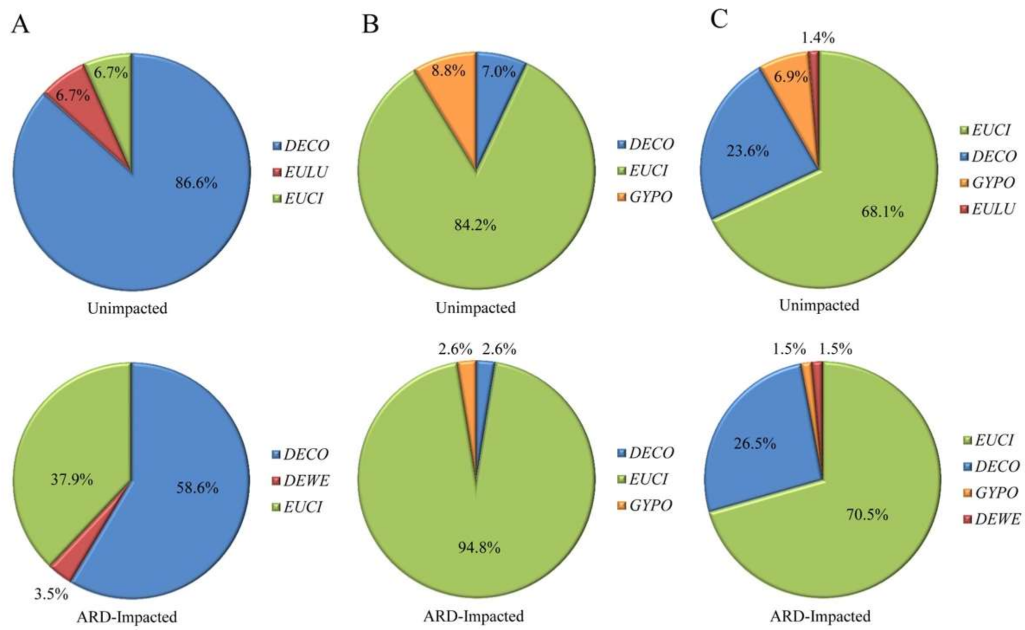

FIG. 3. Percent composition adult (A), larval (B), and total (C) stream salamanders in stream reaches unimpacted and impacted by acid rock drainage in middle Tennessee. Species abbreviations are as follows: DECO – *Desmognathus conanti* (Spotted Dusky Salamander), DEWE – *Desmognathus welteri* (Black Mountain Salamander), EUCI – *Eurycea cirrigera* (Southern Two-lined Salamander), EULU – *Eurycea lucifuga* (Cave Salamander), and GYPO – *Gyrinophilus porphyriticus* (Spring Salamander).

DISCUSSION

We originally hypothesized that salamander diversity measures and relative abundance would be lower in ARD-impacted stream reaches compared to unimpacted reaches and that larvae would be more negatively impacted by disturbance compared to adults. Our findings do not support these hypotheses and suggest that impacts of ARD disturbance on stream salamander assemblages were minimal at the two sites evaluated. Specifically, we found no difference in biodiversity measures (i.e., species richness, diversity, and evenness) between ARD-impacted and unimpacted stream reaches for adult and larval salamanders. Although few other studies have evaluated the impacts of ARD on salamander biodiversity measures, landscape-scale disturbances (such as mountaintop removal mining) result in reduced species richness compared to unimpacted streams (Muncy et al. 2014).

Although we did not find statistically significant differences in relative abundance for larval and adult *D. conanti* and *E. cirrigera* between ARD-impacted and unimpacted stream reaches, *E. cirrigera* adults and larvae tended to compose a greater proportion of adult and larval salamander captures in ARD-impacted reaches compared to unimpacted reaches. Previous research suggests that *E. cirrigera* is somewhat tolerant of stream disturbance and can maintain relatively high abundance in moderately disturbed stream sites (Southerland et al., 2004). Further, stream salamander species that inhabit disturbed stream sites (such as *E. cirrigera*) are defined as "disturbance tolerants" due to their ability to persist in stream ecosystems impacted by moderate anthropogenic disturbance (Surasinghe and Baldwin, 2015).

As we only monitored two sites during this study, continued research at a greater number of ARD-disturbed sites is necessary to better understand the impacts of ARD on stream salamander assemblages. Kucken et al. (1994) observed marked declines in relative abundance of Blue Ridge Two-lined Salamanders (*Eurycea wilderae*) and Blackbelly Salamanders (*Desmognathus quadramaculatus*) in an ARD-impacted stream in the Anakeesta shale formation of the Great Smoky Mountains National Park. Similarly, a fish kill was observed in a stream draining a roadbed fill, which continued for 10 years; downstream of the fill, the pH was acidic (4.5-5.9), whereas pH upstream of the fill was near neutral (6.5-7.0; Huckabee et al. 1975). Stream salamanders are sensitive to changes in stream conditions as evidenced by studies that have evaluated the impacts of agriculture (Willson et al. 2003), urbanization (Barrett and Guyer 2008), and mining (Price et al. 2016). Overwhelmingly, these studies suggest that land-use change in terrestrial areas surrounding stream environments causes increased levels of dissolved sediments and subsequent decline of water quality through increased conductivity and usually large decreases in stream pH (Price et al. 2016, Willson et al. 2003; Huckabee et al. 1975; Barrett and Guyer 2008).

Stream salamanders (i.e., family Plethodontidae) in particular appear to be negatively associated with changes in water quality parameters, perhaps because they rely on cutaneous respiration to acquire oxygen (Wells 2007). This adaptation requires moist environmental conditions for the diffusion of oxygen to occur, which increases the sensitivity of this species group to environmental stressors, especially water quality (Welsh and Droege 2001). For example, Grant et al. (2005) noted that stream salamander abundance was negatively associated with stream acidification across multiple sites in the Shenandoah National Park. We did not document significant differences in pH, dissolved oxygen, or temperature between stream reaches upstream and downstream of ARD disturbance in our study, which suggests that water quality was impacted minimally by ARD disturbance at these sites. Both study sites were mitigated with limestone rock, which has likely attenuated stream pH fluctuations at the sites. Limestone rock additions and drain systems are effective at mitigating pH changes due to acid mine runoff and acid rock drainage (Cravotta and Trahan 1999). Future study efforts at these sites and/or other ARD sites should measure specific conductivity (in addition to other water quality measures) to better understand changes in ion concentrations due to disturbance and to evaluate effectiveness of mitigation measures. In addition, future studies should monitor streams directly after disturbance and for multiple years following disturbance to document the initial impacts and continued long-term changes.

It is essential to monitor streams for anthropogenic impacts to preserve the unique biodiversity present in the southeastern United States, particularly for changes in water quality, which may have negative effects on stream salamander assemblages. Our study provides a preliminary examination of the impacts of rock cuts and ARD on stream salamander assemblages in Middle Tennessee. As we only evaluated the impacts of ARD on salamanders at two sites in Tennessee, and we had limited sampling events at each site, our inference of ARD impacts is limited and should be evaluated on a larger scale (e.g., statewide) to better generalize impacts. Our pilot study data suggests that ARD impacts to stream salamanders were minimal at the two sites we monitored, however, our small sample size makes it difficult to draw larger conclusions. Future studies should include a larger allocation of sample sites at the regional level, multiple ecoregions, and include streams that have a variety of impacts from ARD discharge, including time since disturbance and whether mitigation procedures were implemented. Given the global scale declines of amphibian populations, a better understanding of the primary threats to this vertebrate group is necessary for long-term conservation and management. Alteration of aquatic habitats via anthropogenic land use change represents an acute threat for lotic-dependent amphibian species and continued monitoring is necessary to assess the impacts of these disturbances on stream-dependent organisms.

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