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Remnant Habitat Patches Support Green Salamanders (*Aneides aeneus*) on Active and Former Appalachian Surface Mines

Michael P. Hinkle, Layton A. Gardner, Eric White, Walter H. Smith*[1]*, and Robert D. VanGundy

Department of Natural Sciences, The University of Virginia's College at Wise, One College Avenue, Wise, Virginia 24293, USA
[1]Corresponding author: whs2q@uvawise.edu

***Abstract*.—Surface mining practices associated with coal extraction significantly impact assemblages of amphibians in the Appalachian Mountains; however, the impacts of coal extraction on amphibian habitat associated with rock outcrops is poorly understood. We compared habitat at 45 rock outcrops scattered across the Virginia coalfields to examine if and how habitat features associated with the occupancy of Green Salamanders (*Aneides aeneus*) differ among undisturbed control sites, mined highwalls, and remnant outcrops remaining in small patches of undisturbed habitat on active and former surface mines. An analysis of similarity indicated that the habitat structure of highwalls was significantly different from that of natural outcrops. These habitats did not appear to support populations of Green Salamander, a finding in line with predictions about the impacts of coal extraction on plethodontid salamanders. However, remnant outcrops were not significantly different from natural outcrops with respect to both outcrop structure and surrounding vegetation, despite occurring in highly-fragmented edge habitats located in close proximity to mining activities. We found populations of the Green Salamander at more than 70% of the remnant outcrops, including at sites dominated by invasive vegetation and located within meters of surface extraction activities. Although more work is needed to ascertain the health and status of populations, our data indicate that Green Salamanders occur in small, isolated patches of habitat within a larger disturbed matrix more frequently than previously thought; thus, areas that have been mined may represent an overlooked reservoir of populations potentially crucial to the conservation of the species.**

Key Words.—amphibian; coal; conservation; mining; rock outcrop; Virginia

Introduction

The central Appalachian Mountains of the U.S. are a global hotspot of amphibian biodiversity, with reportedly more than 40 species of amphibians occurring in the region (Powell et al. 2016). Many of these species are plethodontid salamanders adapted to mesic forests and headwater streams, habitats that have been altered throughout the Appalachian Mountains in association with processes used to extract coal (Muncy et al. 2014; Brady 2015). These activities include both less-impactful strip or contour mines and large-scale surface coal extraction in the form of mountaintop removal (MTR) mining, in which overburden is removed from ridgetops and deposited in stream valleys below. Surface mining activities have eliminated more than 4,000 km of headwater streams and have deforested more than 3,000 km^2 of habitat region wide (Saylor 2008; Townsend et al. 2009; U.S. Environmental Protection Agency [USEPA] 2011; Miller and Zegre 2016; Ross et al. 2016).

Coal extraction and subsequent reclamation efforts exert widespread impacts on plethodontid salamander populations. Mountaintop removal mining sites in southeastern Kentucky, USA, show reduced occupancy, abundance, and species richness of stream-dwelling plethodontids (Muncy et al. 2014; Price et al. 2016), a phenomenon associated with large-scale land use trends occurring across entire watersheds and more small-scale changes in microhabitat variables resulting from mining activities (Sweeten and Ford 2016). Although the impacts of coal extraction on terrestrial plethodontids have not received much attention (Wickham et al. 2013), both abundance and species richness are lower on sites formerly mined compared to more pristine sites, likely a consequence of both vegetative removal and soil compaction (USEPA 2003; Williams 2003; Wood and Williams 2013). In other cases, reforested surface mines can provide suitable habitat for some assemblages of terrestrial salamanders, provided that nearby streams have not experienced impaired water quality (Brady 2015).

Among terrestrial salamanders in the central Appalachian coalfields, the Green Salamander (*Aneides aeneus*) is of particular concern. The center of distribution of this species overlaps with the central Appalachian coalfields, and its status as an arboreal cliff

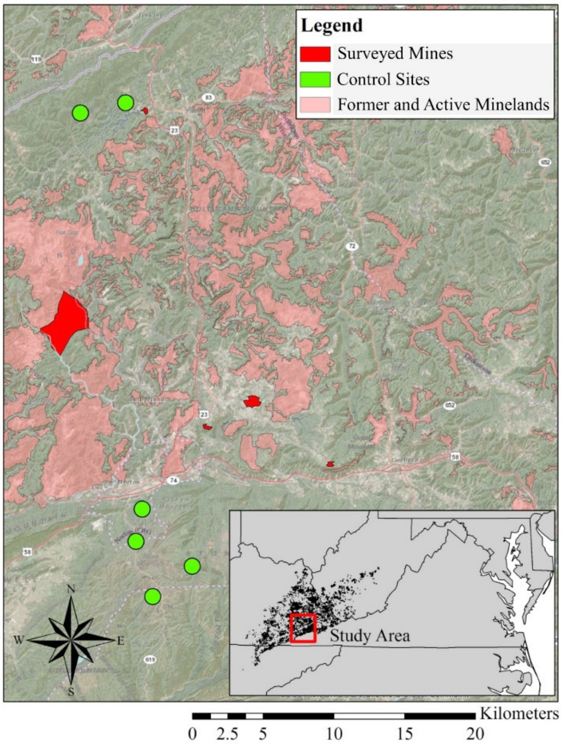

FIGURE 1. Location of surface mines and control sites where we surveyed for Green Salamanders (*Aneides aeneus*) in Wise County, Virginia, USA. Black polygons on the inset map denote areas impacted by surface mining.

specialist places it in habitats (vertical rock outcrops and surrounding forests) that are often destroyed or substantially altered during mining activities. Habitat loss is one of several potential causes for past declines in populations of Green Salamanders (Corser 2001). In addition, this species is rare across its range, occurring in isolated populations that are vulnerable to anthropogenic disturbance (Petranka 1998; Pauley and Watson 2001). Accordingly, six states provide special protected status to Green Salamanders, and the species is under consideration for federal listing (U.S. Fish and Wildlife Service [USFWS] 2015).

Green Salamanders are assumed to be sensitive to mineral extraction activities and absent from areas that are either mined or nested within large mine complexes (Felbaum and Mitchell 1995; Giese et al. 2012; Kiviat 2013); however, we are aware of no work that has specifically addressed the impacts of mining-related disturbance on Green Salamanders or their associated rock outcrop habitats. Here we compare habitat used by the Green Salamander from mined and unmined areas of southwest Virginia, USA. We address two primary questions: do mining activities alter rock outcrop and forest habitat variables known to influence occurrence of Green Salamanders; and do Green Salamander populations occur on sites that have formerly experienced intensive surface mining activities?

Materials and Methods

Site selection and habitat characterization.—Beginning in fall 2015, we randomly selected 45 rock outcrops from a group of five active or former surface mines in Wise County, Virginia, USA. Wise County is centered in the Virginia coalfields and is the most heavily surface-mined county in that state (Li and Zipper 2015). We surveyed three older (pre-1977), unreclaimed contour mines along the south slope of Coeburn Mountain, one former, 160-ha surface mine encompassing the headwaters of Yellow Creek containing both large-scale surface mining (circa 1990) and older contour (pre-1977) mine features, and one active mountaintop removal surface mine on Divide Ridge (Fig. 1). Rock outcrops at all sites included artificially created highwalls (vertical rock surfaces created as a result of mineral extraction; n = 14; Fig. 2A) and natural rock outcrops remaining on isolated, small (generally < 5 ha) patches of undisturbed forest habitat nested within the context of the surface mine (n = 18; Fig. 2B). We hereafter refer to these latter outcrops as remnant outcrops.

We also surveyed a third series of nearby, randomly selected rock outcrops (n = 13) from undisturbed hardwood forest on High Knob in the Jefferson National Forest as control sites (Fig. 2C). We chose our control sites from this area because of its proximity to our mined outcrop sites (within 10 km) and its location in the same physiographic context (Appalachian Plateau Physiographic Province). This area has also not been subjected to recent (within the past 50 y) anthropogenic disturbance, and previous survey work documented the occurrence of Green Salamanders (Smith et al. 2015). All outcrops we chose as study sites were of Pennsylvanian sandstone and occurred at similar elevations (750–850 m above sea level). Furthermore, all outcrops were erosional remnants within ridgetop or upslope forests, contained a single exposed rock face jutting into the forest, and were < 10 m in length (mean length = 7.4 ± [SD] 0.6 m). Outcrops were also generally < 5 m in height (mean height = 4.6 ± 2.6 m).

We tested the hypothesis that both highwall and remnant sites possessed significantly different outcrop and forest habitat characteristics than control sites as a result of mining activities. At each outcrop, we measured a series of variables related to crevice refugia in the outcrop itself and the context of the outcrop with respect to the surrounding forest. These variables included height, width, depth, and distance above ground of each accessible crevice within the outcrop, the aspect and slope at the outcrop, nearest distance from the outcrop to the forest edge, and the canopy cover, litter depth, tree density, and proximity of trees to the outcrop within 100 m^2 of the outcrop. We predicted that highwalls

Figure 2. Examples of habitat on mined and unmined sites. (A) a mountaintop removal mining site, with exposed highwall escarpments visible below the horizontal mine bench in the background. (B) a salamander-occupied remnant outcrop in a small patch of remaining habitat within a larger surface mine, with several coauthors pictured for scale. Cleared habitat on the mine begins behind the outcrop, wraps around to the right and left of the outcrop, and continues a short distance behind the photographer. (C) a control site within undisturbed habitat in the Jefferson National Forest. (Photographed by Walter H. Smith).

and remnant outcrops would have significantly less vegetation in close proximity to the rock face and fewer available crevice refugia than control outcrops as a result of mineral extraction activities.

We selected variables based on those associated with Green Salamander occupancy in past work at our control sites (Smith et al. 2017), with habitat data collection protocols for each habitat variable identical to those used in this aforementioned work. We analyzed crevice dimensions by determining the maximum height, width, and depth of each crevice, with depth measured as the maximum distance that a flattened wooden probe could be inserted into the crevice, per the methodology of Rossell et al. (2009). We measured the height of each crevice above ground as the distance between the ground and lowest point of the crevice opening. We measured all accessible crevices on each outcrop and averaged values for each measurement per outcrop. We measured the aspect (azimuth) of each outcrop at the rock face, with slope determined using a Suunto clinometer (Suunto, Vantaa, Finland).

We measured variables related to forest context using a 100 m^2 plot (10 × 10 m) centered on each outcrop. We chose this plot size due to (1) the typical dispersal distance of Green Salamanders as observed in previous work in our study region; (2) our ability to maximize the number of independent, non-overlapping plots at each site; and (3) the identification of variables within this plot size as being associated with Green Salamander occupancy by Smith et al. (2017). We measured litter depth at 1 m increments within this plot, with canopy cover measured using a GRS densitometer (Geographic Resource Solutions, Arcata, California). We averaged measurements for each variable for each plot. We also counted the standing trees within each plot and recorded the straight-line distance of each tree to the rock face. We determined the mean distance of tree-to-outcrop proximity measurements for each plot.

Salamander survey protocols.—In addition to habitat comparisons, we also examined if Green Salamanders were present in mined highwalls or natural escarpments remaining on surface mines. We therefore performed time- and area-constrained surveys of each rock outcrop and highwall in June and July (a peak local time for surface and breeding activity) 2016, searching all available crevices and substrate (rock surfaces, trees) within 10 m of the outcrop with an LED headlamp for 30 min or until all available substrate was searched. We visited each site on two occasions during the study period, spacing visits at least two weeks apart. This approach has reliably detected the presence of Green Salamanders at our control sites and other locations within this study area in past work (Smith et al. 2015; Smith et al. 2017). We avoided performing surveys within 24 h of a rainfall event, due to the association of recent rainfall with lower detection of Green Salamanders (Smith et al. 2017). Because we were working on active mines with safety concerns and nocturnal access restrictions, we only performed diurnal searches at each site. As a result, we consider our salamander survey data to be indicative only of the presence of Green Salamanders rather than population abundance or the definite absence of the species from a site.

Statistical analyses.—We performed multivariate comparisons to examine differences in outcrop habitat between our mined, remnant, and control outcrop sites. We imported all habitat variables across all sites into the Primer v7 software package (Clarke and Gorley 2006). We then built a resemblance matrix (Euclidean distance) representing dissimilarity in habitat variables across all pairwise combinations of sites. We normalized data prior to analysis. We then used a non-metric multidimensional scaling analysis (nMDS) to visualize differences between these sites, along with an Analysis of Similarity (ANOSIM; Clarke 1993) to test

Table 1. Habitat characteristics of highwall (n = 14), remnant (n = 18), and control (n = 13) outcrops from five active and former surface mines and control sites in Wise County, Virginia, USA. Values reflect means across sites ± 1 SD. Units of characteristics are azimuth for Aspect, degrees for slope, percentage for Canopy Cover, meters for Distance to Forest Edge, and centimeters for all other characteristics.

Outcrop Type	Aspect	Slope	Crevice Height	Crevice Width	Crevice Depth	Distance Above Ground	Canopy Cover	Litter Depth	Tree Count	Distance to Trees	No. of Crevices	Distance to Forest Edge
Highwall	176.8 ± 179.7	6.2 ± 6.3	5.98 ± 6.41	12.50 ± 13.39	4.79 ± 5.13	44.94 ± 48.15	61.0 ± 40.5	2.05 ± 2.03	4.4 ± 4.5	603.1 ± 621.6	0.93 ± 0.99	—
Remnant	130.2 ± 107.4	21.6± 17.6	18.0 ± 21.8	40.4 ± 19.5	14.9 ± 9.2	94.7 ± 46.7	94.5 ± 8.0	3.9 ± 1.1	9.5 ± 3.9	433.8 ± 138.2	5.6 ± 2.8	5.2 ± 2.0
Control	187.4 ± 105.5	23.5 ± 26.6	10.5 ± 10.9	39.9 ± 28.6	14.9 ± 5.2	115.5 ± 36.0	100.0 ± 0.0	3.5 ± 1.1	8.0 ± 3.0	459.2 ± 192.3	3.5 ± 1.3	83.1 ± 49.5

for differences in habitat similarity between highwall, remnant, and control outcrops. We chose our outcrops and associated forest plots to be as spatially independent as possible and separated by a distance greater than that typically observed for dispersing Green Salamanders in past work within our study region (Smith et al. 2017). However, multiple outcrops were present at each mined or control site and were therefore not completely independent. As a result, we used a nested ANOSIM design to compare similarity across types of outcrops while accounting for the influence of individual sites. We assessed significance (α = 0.05) using 9,999 permutations of our dissimilarity matrix. We then followed this analysis with a similarity percentage analysis (SIMPER; Clarke 1993) to determine which habitat variables were responsible for any observed differences between types of outcrops. Because our mined sites contained those exposed to two different mining practices (contour versus MTR mining), we also performed an ANOSIM to test for a difference in mined outcrop habitat across these two different types of sites. Because this resulted in multiple analyses being run on the same dataset, we performed a Bonferroni correction on resulting *P*-values to account for the influence of these multiple comparisons. We expected that we were more likely to detect Green Salamanders in control sites as opposed to mined sites, where we expected the species to be absent. We used an Exact Goodness-of-Fit Test (α = 0.05) to compare these expected counts for each type of outcrop to the number of outcrops within each type where salamanders were encountered during field surveys.

Results

Outcrops on mined and control sites exhibited substantial differences in both outcrop habitat and the forest context surrounding each outcrop (Table 1). Specifically, highwall-associated outcrops contained fewer crevices, occurred above less steep slopes, and contained less vegetation adjacent to the outcrop than both remnant and mined sites. Multivariate analyses mirrored these cursory comparisons. There was a significant difference between outcrop types (Global R = 0.29, P = 0.020; Fig. 3), with highwall outcrops significantly different than both remnant outcrops and control outcrops (Adjusted $P < 0.05$ in post-hoc, pairwise comparisons). There was not a significant difference in rock outcrop habitat on differing sites, considering all outcrop types (Global R = 0.105, P = 0.340).

Differences in outcrop habitat structure were driven by available vegetation and crevices at varying types of outcrops (Table 2). Specifically, highwalls contained less canopy cover, fewer trees at greater distances

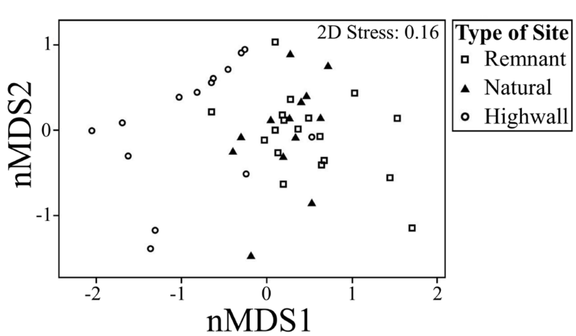

Figure 3. Non-metric multidimensional scaling (nMDS) plot depicting variation in habitat variables found across highwall, remnant, and natural (control) outcrops. The distance between symbols indicates similarity in habitat across sites.

Table 2. Similarity percentage (SIMPER) results, showing percent contributions of habitat variables to observed differences between mined highwalls and natural outcrops.

Habitat Variable	Percentage Contribution
Number of Crevices	12.79
Canopy Cover	11.46
Number of Trees	10.61
Litter Depth	10.35
Crevice Depth	9.92
Distance to Trees	8.85
Distance of Crevice Above Ground	8.65
Crevice Height	8.37
Crevice Width	7.90
Aspect	6.10

from the outcrop, and fewer crevices than remnant and control sites. These three variables together accounted for 34.9% of the observed differences between highwalls and natural outcrops. Outcrop habitat was not significantly different between contour and MTR mines (Global $R = 0.007$, $P = 0.664$).

Our salamander survey data did not support the prediction that Green Salamanders would be present on all control outcrops but not located at any highwall or remnant outcrops (Exact Test; $P < 0.001$). Instead, we found Green Salamanders present at all control outcrops and at 13 (72%) of our remnant outcrops. We additionally found evidence of reproduction (females guarding egg clutches, presence of younger juveniles) at 10 control (77% of all sites) and nine remnant (50% of all sites) outcrops. We found no Green Salamanders present at any outcrops associated with mined highwalls.

Discussion

We found evidence that both Green Salamanders and their associated habitats can occur within active and former surface mines given certain criteria. This was true for both active surface mines and former mines that have remained in an overall degraded state of habitat quality for four decades. We specifically found that while highwall habitats contained significantly different habitat structure on mined sites as opposed to control outcrops, remnant outcrops remaining on active and former surface mines did not differ significantly from control sites. On the surface, it is not surprising that highwalls would contain different habitats from our other two site classifications. Unreclaimed escarpments erode at different rates from natural cliffs and bluffs, and it may take decades before mature forest structure is established around a highwall following mining (Bell et al. 1989; Kumar and Sweigard 2011). Artificial escarpments should therefore be significantly different from natural cliffs and bluffs in mature forest in terms of habitat structure, and our habitat analyses supported this prediction.

By contrast, it is surprising that remnant outcrop habitat did not differ significantly from outcrops nested within intact, relatively pristine hardwood forests at unmined control sites. Our results showed that these outcrops were indistinguishable from unmined control habitats, at least in terms of outcrop morphology and forest structure characteristics associated with Green Salamander occupancy (Smith et al. 2017). We did not, however, measure microclimate variables, such as temperature or relative humidity, that may be altered in disturbed vegetative buffers adjacent to rock outcrops (Petranka 1998). Regardless, our structural habitat data suggest that Green Salamanders may still be able to occupy such remnant outcrops, given their similarity to control sites harboring the species.

We encountered Green Salamanders at nearly three quarters of our remnant outcrops, despite their apparent absence from all highwall sites. The apparent absence of salamanders from highwalls follows from our habitat comparisons because our highwall sites contained significantly less canopy cover, nearby vegetation, and available crevices as compared to control or remnant sites. Green Salamander occupancy is associated with both crevice depth and the density and proximity of adjacent vegetation in natural outcrops (Waldron and Humphries 2005, Smith et al. 2017), meaning that highwalls largely lacking available crevices and nearby vegetation should be unlikely to harbor Green Salamander populations. These data also support the removal of forest ecosystems and rock outcrops and their subsequent replacement with highwalls and other mine features as a possible factor in driving the localized extirpation of Green Salamander populations across the central Appalachian region.

Our finding that Green Salamander populations do occur at remnant outcrops remaining on both active and former surface mines suggests that this species may be more resilient to disturbance than previously thought. We not only found evidence of the presence of Green Salamanders at our remnant outcrops but also found evidence of active reproduction and multiple age classes at several sites, suggesting that the presence of these populations was not simply the result of a small number of older adults remaining following initial disturbance from mining activities. These results pose several new questions relevant to dispersal capabilities and response to disturbance by Green Salamanders. For example, are Green Salamanders able to exist in these small, isolated remnant habitats within the context of larger, non-forested surface mines without substantial dispersal and immigration from nearby populations, or is this species able to effectively disperse across suboptimal habitat to recolonize remnant outcrops and/or buffer populations against local extinction more than has previously been thought? Others have suggested that Green Salamanders may indeed be capable of at least limited dispersal across disturbed habitats such as cleared forests (Riedel et al. 2006) and roads (Williams and Gordon 1961; Cupp 1991), although future work will need to investigate these phenomena in greater detail to more fully ascertain the response and resilience of this species to habitat fragmentation and disturbance.

Although our results suggest some promise for Green Salamander conservation in the Appalachian coalfields, we acknowledge that appropriate inferences from our results are limited to the presence of the species alone. Population health is clearly reliant on a host of other factors, including but not limited to abundance, age structure, and gene flow or other population genetic parameters, and many populations on surface mines are likely small, isolated, and potentially experiencing or susceptible to impacts from inbreeding and disease or other environmental stressors. These factors, coupled with the amount of suitable habitat destroyed by surface mineral extraction on large mines, almost certainly make surface mining activities result in net negative impacts on Green Salamander populations, despite the potential resilience of some populations. These questions will be crucial for future researchers investigating the health and status of Green Salamander populations on former and active mines.

Regardless, our findings suggest that former and active mines in the Appalachian coalfields serve as potential reservoirs of Green Salamander populations that may be crucial for regional conservation efforts. Locally, the new populations located through this study helped to connect two clusters of known populations in Virginia that were previously considered to be highly disjunct (Smith et al. 2015), filling a distributional gap between populations on the Appalachian Plateau and Valley and Ridge Provinces. This may be a similar case for other portions of the Appalachian coalfields where surveys on or near surface mines have not taken place, and remnant outcrops on both active and former surface mines should therefore be prioritized for species inventories and habitat protection. In addition, ongoing work in the coalfields is centered around restoring native forest structure on former surface mines (Zipper et al. 2011). Future, more intensive work on isolated, remnant populations of Green Salamanders may be able to identify best practices for mine restoration that buffer known salamander populations against local extinction and restore or enhance connectivity between them.

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Michael Hinkle received a B.S. degree from the University of Virginia's College at Wise (UVa-Wise), USA, in 2017. During his time at UVa-Wise, he researched the impacts of surface mining on the Green Salamander in southwestern Virginia. He is now an environmental interpreter at Natural Tunnel State Park, Virginia, USA. (Photographed by Michael Hinkle).

Layton Gardner received a B.S. degree from the University of Virginia's College at Wise, USA, in 2017. He participated in research while an undergraduate, including habitat analysis and health of the Green Salamander (*Aneides aeneus*), and defining microclimate gradients in central Appalachia. (Photographed by Ashlee Taylor).

Eric L. White received a B.S. from the University of Virginia's College at Wise, USA, in 2017, where he performed research on the Green Salamander (*Aneides aeneus*). He is now a Graduate Research Assistant at North Carolina State University, Raleigh, USA, pursuing a M.S. in Fisheries, Wildlife, and Conservation Biology. His research focuses on using small unmanned aerial vehicles to identify and target non-cropland weed species. (Photographed by James M. Brooke).

Walter H. Smith is an Assistant Professor of Biology at the University of Virginia's College at Wise, USA. He earned his Ph.D. in Biological Sciences at the University of Alabama, Tuscaloosa, USA, in 2011. His research focuses on the overlap between amphibian conservation and socioeconomic change in central Appalachia. (Photographed by Katie Dunn).

Robert D. VanGundy is a Geology Instructor at The University of Virginia's College at Wise, USA. He has interests in climate, karst hydrogeology, and geologic influences on mined lands and karst environments. He earned his M.S. in Geology at the University of North Carolina, Chapel Hill, USA, in 1983 and worked in offshore exploration for Shell Offshore, Inc. prior to entering academia. (Photographed by Bob VanGundy).