

# Seasonal Variation in Salamander Abundance and Species Diversity in Relation to Distance from a Trail

Sydney Stahl<sup>i</sup> & Jennifer Deitloff<sup>i</sup>

Amphibian populations are shown to decline when humans are present. Human influence affects food and refuge availability, and habitat quality. Urbanization is one of the biggest anthropogenic drivers of habitat fragmentation. Habitat fragmentation and urbanization reduce genetic variation by creating barriers between populations and altering environmental conditions. The most prominent form of fragmentation that humans cause is the creation of roads. Population size of amphibians decreases as density of roads increases. Lower soil moisture and limited refuge are linked to this decrease. While road effects on amphibians are observed to be negative, the effects of trail are relatively unknown. Creation of trails is less intrusive than roads but could impact habitats similarly. This study focuses on the abundance and variation of natural salamander communities observed through plots perpendicular to a trail. We examined transect plots perpendicular to the trail, searching leaf litter and cover objects (rocks and logs) for salamanders. To account for seasonal variation, this study was conducted in both the fall and spring. We recorded species, snout-vent length, age class, and sex to determine variation as well as abundance. We saw no significant difference in salamander abundance or variation found closer or further from the trail. Therefore, our study does not support the hypothesis that trails negatively affect salamander density, abundance, or diversity. Hiking trails having minimal impact on salamander abundance and diversity contributes to the growing knowledge on the depth of disturbance humans may have within natural environments.

The presence of humans in natural environments can negatively impact amphibian populations. Urbanization, the increase in the proportion of people living in towns or cities<sup>1</sup>, can fragment amphibian habitats and have negative impacts on ecological systems.<sup>2</sup> An area is considered urbanized when human population density reaches a minimum of 50,000 people within a continuously built-up area.<sup>3</sup> Less impactful types of urbanization that are less destructive can include construction zones, the presence of roads in rural areas, and recreational use such as hiking or mountain biking on constructed trails.<sup>4</sup> In addition to disturbance, human activities also impact food and refuge availability for amphibians.<sup>5</sup>

Urbanization introduces physical barriers, such as roads, that fragment natural habitats, decreasing connectivity and gene flow, which decreases overall genetic diversity.<sup>6</sup> However, disconnected populations are not affected at a smaller level, compared to overall population levels where gene flow is restricted.<sup>7</sup> Fragmented populations are isolated from each other, and compared to a historically larger, connected population, these smaller populations experience genetic drift, lower genetic diversity, or increased rates of inbreeding.<sup>8</sup> Limited gene flow leads to a diminished potential for adaptation to new environmental conditions.<sup>9</sup> The barriers introduced from urbanization are directly correlated to lower dispersal patterns and lead to limited reproduction.<sup>10</sup> Therefore, urbanization has a negative influence on amphibian populations through habitat fragmentation and genetic isolation.

Fragmentation of amphibian habitat can occur through the construction of roads that can impact surrounding amphibian populations through active avoidance of roads and increased mortality that results in roadkill.<sup>11</sup> Amphibians and reptiles are most likely to exhibit population declines associated with road mortality compared to other groups of vertebrates.<sup>12</sup> Amphibian population size also decreases as the density and traffic intensity of roads increases.<sup>13</sup> Roads fragment pond-breeding amphibian populations which reduces genetic diversity and leads to smaller population sizes.<sup>14</sup> New roads reduce individual movements of amphibians at a faster rate than overall population dynamics.<sup>15</sup> Edge effects are also introduced with the construction of roads and lead to changes in population or community structure that occur at the boundary of two or more habitats.<sup>16</sup> Edge effects include both abiotic and biotic influences on populations near forest boundaries.<sup>17</sup> These effects can lead to changes in species' abundance and community structure.<sup>18</sup> Ninety-six percent of Neotropical amphibians showed an edge response to fragmented forest landscapes, with 74.5% decreasing in proximity to forest edges.<sup>19</sup> While all amphibian populations are affected, salamanders are more sensitive to edge effects than anurans.<sup>20</sup>

Urbanization negatively affects natural systems by changing the structure and function of ecosystems. For example, urbanization changes riparian forest ecosystems by modifying stream morphology, increasing run-off and soil erosion, removing trees, adding chemical pollutants, and decreasing ground cover.<sup>21</sup> Changes to habitats within an ecosystem affect

<sup>i</sup> Commonwealth University-Lock Haven

all organisms that rely on and use them, including salamanders. For example, conversion of riparian forest to urban land can result in the decline of populations of southern two-lined salamander, *Eurycea cirrigera*, by an estimated 44%,<sup>22</sup> and populations of dusky salamanders, *Desmognathus fuscus*, decreased when exposed to high levels of urbanization.<sup>23</sup> Construction of roads harms amphibian habitat quality through decreasing the surrounding soil moisture.<sup>24</sup> Salamanders require damp habitats to maintain moisture on their skin, which regulates many physiological processes.<sup>25</sup> Roads can increase the rate of water vapor loss from soil through evaporative heating, introducing debris and dirt, and reducing abundance of cover objects.<sup>26</sup> When a road is constructed, the area is cleared at the location as well as adjacent areas to store large machinery. This removes necessary cover objects such as rocks and logs that salamanders use to seek refuge. Lack of cover objects and decreased soil moisture reduces the success of an amphibian population.<sup>27</sup> Ultimately, the presence of humans reshapes natural ecosystems, making them unfavorable to salamander populations.

Although roads decrease amphibian density and deter habitat quality,<sup>28</sup> the impact of hiking and mountain biking trails is not well understood. Trails are like roads because they potentially increase fragmentation and decrease habitat quality and there are few studies addressing the impact of trails specifically regarding salamander diversity and abundance. In some situations, salamanders are found in higher abundances closer to the trail.<sup>29</sup> It is unclear whether this higher abundance is due to the trail having no effect on salamander distribution, or an increase in microhabitat availability with rocks and logs near edges of the trail.<sup>30</sup>

*Plethodon cinereus*, the Eastern Red-backed Salamander, has a broad distribution throughout the Eastern United States.<sup>31</sup> They are most commonly found within forests with greater percent canopy cover, larger trees, and higher densities of well-decayed wood debris. These factors provide optimal moisture with a cool microclimate for *P. cinereus*.<sup>32</sup> Like other species in the Plethodontidae family, *P. cinereus* use cutaneous respiration for gas exchange. To retain a level of dermal moisture and breathe, plethodontids rely on moist microhabitat conditions.<sup>33</sup> The local distribution of *P. cinereus* results from the suitability of microhabitat features as well as the habitat's ability to meet physiological requirements.<sup>34</sup> They are commonly found under cover objects, such as rocks and logs, and within leaf litter on the forest floor where they seek refuge in moist soils or away from the dry air.<sup>35</sup> In addition to providing moisture, cover objects and leaf litter provide an escape from predators.<sup>36</sup> *Plethodon cinereus* will burrow into soil and leaf litter to seek optimal temperatures.<sup>37</sup> During the warmer months, they are usually found near the surface of soil, and during colder months are found deeper in soils.<sup>38</sup> Therefore, there is a seasonal variation in the depth distribution of surface level where *P. cinereus* are found. To address seasonal variation, data collection took place in both the spring and fall seasons. We conducted this study to address questions about how salamander density changes when hiking trails are present. We hypothesized that salamander abundance will increase as

distance from the trail increases due to improved habitat quality and reduced disturbance away from trail edges.

## Methods

We sampled locations in central Pennsylvania during both spring and fall seasons (Spring 2023 and Fall 2023) to address seasonal variation. For the spring season, we assessed recreational trails at McElhattan Run, Gottshall Run, and Gamble Run. For the fall season, we assessed McElhattan Run, Gottshall Run, and Lick Run. We chose to not survey Gamble Run in the fall because no salamanders were found at this location in the spring; instead, we chose a new location in the fall, Lick Run (Table 1).

In general, at each location, we created three plots using measuring tapes on the trails, except at Lick Run where we were unable to consistently survey for 50m. For each plot, we measured 20 meters along the trail and 50 meters perpendicular to it, to create a 20x50-meter plot (Figure 1). For each plot, we performed transect searches by starting at the trail on the 0, 5, 10, 15, and 20 meter marks, and walking toward the 50-meter boundary of the plot, searching the entire surface of the forest floor or creek bed. For Lick Run, PA, we were unable to conduct 50 meter transects since the landscape was unfavorable (edge of a mountain and a wider stream). The maximum distance we measured perpendicular to the trail was 31 meters. For all locations (including Lick Run), we searched under every cover object (rocks, logs, etc.) and leaf litter for salamanders. When a salamander was found, we recorded the species, snout-vent length (SVL), age class, sex, and distance from the trail. We determined age class using a SVL standard of 34mm for adults, and anything less than that was classified as a juvenile.<sup>39</sup> When determining the sex of an individual, we used candling.<sup>40</sup> Males were identified through the presence of vas deference, gravid females were identified through the presence of eggs, and nongravid females were identified through the absence of both these structures. After this data was collected, we placed the moved object in its original configuration on the forest floor and returned each salamander to the precise location where it was found.

We used a correlation analysis to examine the association between salamander abundance and trail distance at each location separately. Seasons were combined into one analysis for locations with data from both spring and fall. We performed this correlation analysis in R v4.0.3. We also included summary figures of each location and collecting period to examine (1) differences among species composition and abundance and (2) differences among sexes and age classes.

## Results

For each plot that was surveyed in both the spring and fall season at McElhattan Run, PA, we found a positive, non-significant correlation with more salamanders found as distance from the trail increased ( $r = 0.35$ ,  $p = 0.32$ ; Figure 2). Within the landscape of McElhattan Run, there is a stream that runs through the latter half of the plot which could be the cause

of the higher abundance at approximately 35-40 meters. At Gottschall Run, PA, we found no correlation between salamanders found and distance from the trail for both spring and fall seasons ( $r = 0.087$ ,  $p = 0.81$ ; Figure 2). No salamanders were found at Gamble Run, PA. Finally, at Lick Run, PA, we found no correlation between salamanders and distance from the trail during the fall only ( $r = -0.42$ ,  $p = 0.23$ ; Figure 2). We also performed correlation analyses for seasons separately at McElhattan Run, PA and Gottschall Run, PA and found non-significant results (results not reported).

Locations varied in the species found as well as in abundance of juveniles, males, and females. *Plethodon cinereus* was the most abundant species found at all three locations (Figure 3). *Eurycea bislineata* and *Desmognathus fuscus* were only found at McElhattan Run, PA, and *Notopthalmus viridescens* was only found at Gottschall Run, PA (Figure 3).

Overall, we found more juveniles than adults at all three locations (Figure 4). Juveniles were found in the highest abundance at Gottschall Run, PA (Figure 4). In addition, we found more gravid females at McElhattan Run, PA than the other two locations (Figure 4). Comparing all three locations, we found more males and nongravid females at McElhattan Run, PA (Figure 4). We found more salamanders overall during the fall than the spring (Figure 3 and 4).

## Discussion

We examined the distribution and species variation of salamanders surrounding recreational hiking trails in central Pennsylvania. We hypothesized that when trails were present, salamander abundance will increase as distance from a trail increases. When we conducted transect plots perpendicular to trails to survey salamander populations, we found no clear patterns showing recreational hiking trails influence salamander diversity or abundance. In general, salamander diversity, abundance, and distribution neither increased nor decreased in the presence of hiking trails assessed. While Davis (2007) and Fleming et al. (2011)<sup>41</sup> reported that trail maintenance increased microhabitat abundance and sometimes salamander numbers, our results did not show a similar pattern, suggesting that the effects of trails may vary by habitat context or trail design. Similarly, our results indicate no preliminary correlation between a specific age class and proximity to a trail.

While roads decrease amphibian density and habitat quality,<sup>42</sup> existing forest roads may vary in impact on forest animal species.<sup>43</sup> Salamanders within riparian forests were reduced along both active and abandoned logging roads<sup>44</sup>, and *P. cinereus* was observed to be less abundant near gravel road edges.<sup>45</sup> Ungated forest roads are typical roads with vehicular traffic, but gated forest roads have minimal vehicle traffic and are equivalent to human-made trails. The main difference between gated and ungated forest roads is vehicle traffic, which is likely an important factor in determining edge effects on salamanders.<sup>46</sup> Gated forest roads were shown to be less harmful with no detectable effects to terrestrial salamanders than ungated forest roads.<sup>47</sup> Trails assessed within this study

lacked vehicular traffic, and we found no significant correlation between salamander abundance and distance from a trail. This adds evidence to support vehicle traffic's impact on salamander abundance since trails with little to no vehicle traffic seem to have minimal influence on salamander populations compared to roads and trails with vehicle traffic.

Riparian forest conversion to urban land results in significant decline of salamander population abundance.<sup>48</sup> Additionally, streams with a history of timber harvesting and isolation from other bodies of water showed reduced salamander populations.<sup>49</sup> Salamanders are frequently found around healthy forested streams since moist, cool environments are available.<sup>50</sup> Within the McElhattan Run site, there was a stream that ran through the latter portion of transect plots surveyed (approximately 35-40 meters). We found a higher abundance of salamanders around this stream within each respective transect plot of McElhattan Run. There were no streams present at the other locations studied (Gottschall Run and Lick Run), though interestingly, a high abundance of salamanders was found for the same distance from the trail in Gottschall Run, PA as McElhattan Run. This poses the question of the stream's influence on salamander dispersal within each respective plot. It would be interesting to examine whether trail presence or stream presence have a greater influence on overall salamander abundance.

Urbanization and human influence occur on a spectrum in natural ecosystems. Roads are known to harm amphibian populations through direct mortality, and indirectly, via altering the surrounding habitat conditions.<sup>51</sup> Recreational hiking trails could have a similar effect to roads by decreasing habitat quality and increasing fragmentation. Edge effects have negative impacts on natural animal populations in forested areas. In this study, we found no evidence to suggest that recreational hiking trails influence salamander populations. Previous studies found similar results where salamander populations were not influenced by the presence of recreational trails. However, maintained trails alter microhabitat availability, impacting the distribution of salamanders.<sup>52</sup> Salamanders are also indicators of environmental quality due to their sensitivity to changes in temperature and water quality.<sup>53</sup> Awareness of the potential impact of hiking trails on salamander populations allows for an overall understanding of ecosystem health. While our results did not find evidence that recreational hiking trails negatively impacted salamander populations in our study sites, we caution that these findings may not generalize to all regions or trail types. Continued monitoring across varied habitats and trail designs is important to confirm these patterns. This benefits all ecological systems through increased awareness of the environment and conservation 🦎

## Acknowledgements

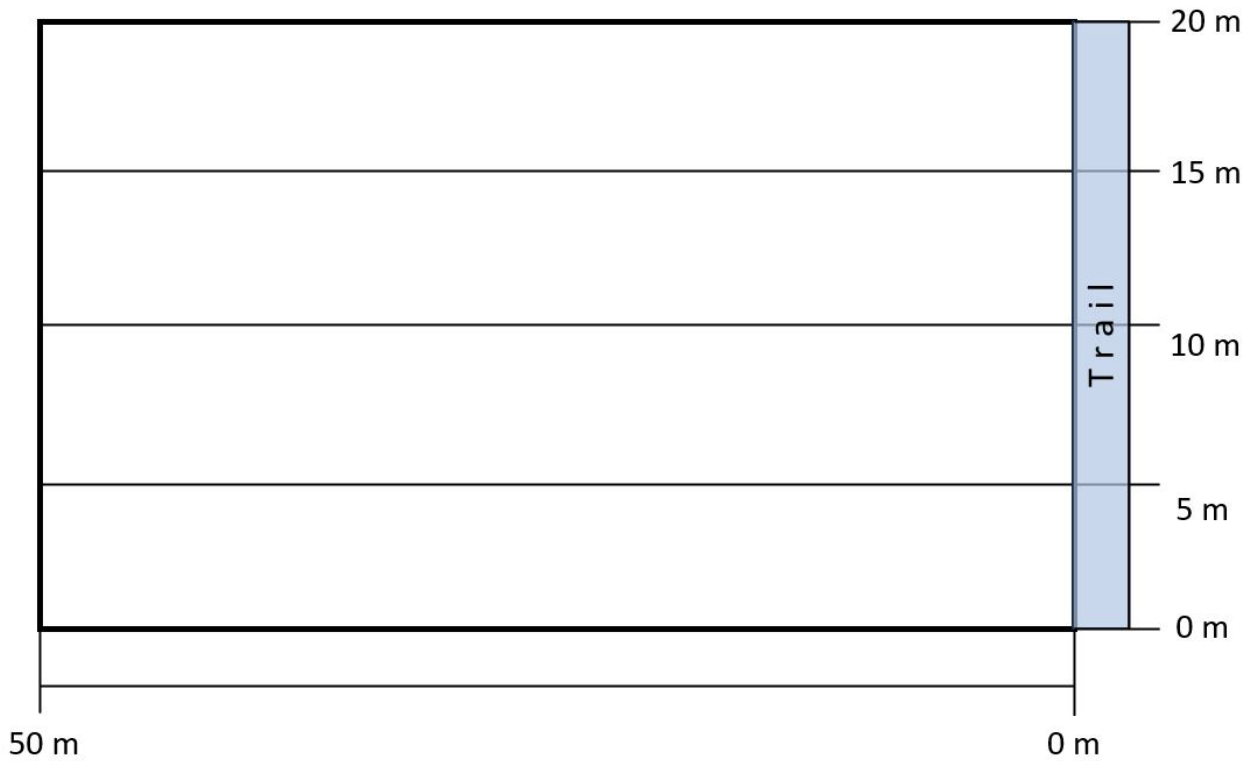
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**TABLE 1.** Locations of study recreational trails.

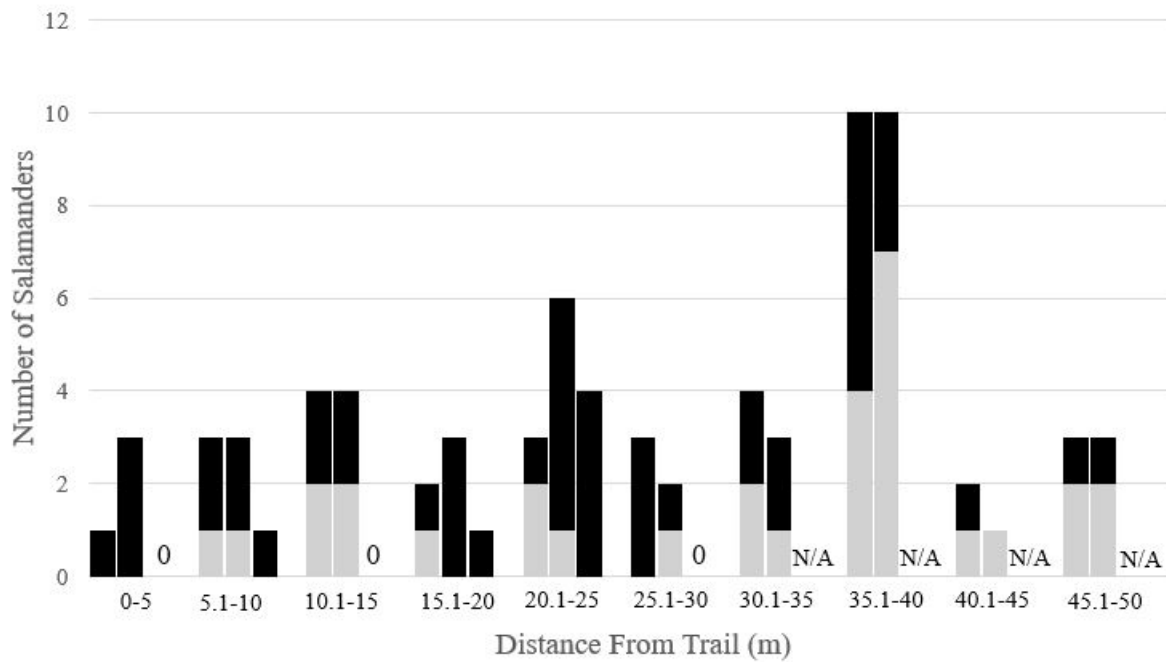
Trail Locality	Location	Season	Date	Plot Coordinates
McElhattan Run	Wayne Township, Clinton County, PA	Spring	5/11/2023	Plot 1: 41.134157, -77.340066
			5/20/2023	Plot 2: 41.133624, -77.339519
	Fall	5/20/2023	Plot 3: 41.132856, -77.339004	
		10/8/2023	Plots 1-3	
Gottschall Run	Crawford Township, Clinton County, PA	Spring	5/31/2023	Plot 1: 41.082493, -77.272994
				Plot 2: 41.082404, -77.270719
	Fall	9/10/2023	Plot 3: 41.082905, -77.267254	
				Plots 1-3
Gamble Run	Watson Township, Lycoming County, PA	Spring	5/25/2023	Plot 1: 41.246952, -77.355373
				Plot 2: 41.247823, -77.357293
Lick Run	Farrandsville, Clinton County, PA	Fall	10/23/2023	Plot 1: 41.185649, -77.513610
				Plot 2: 41.186360, -77.514157

**FIG 1.** Template of transect layout for each plot.



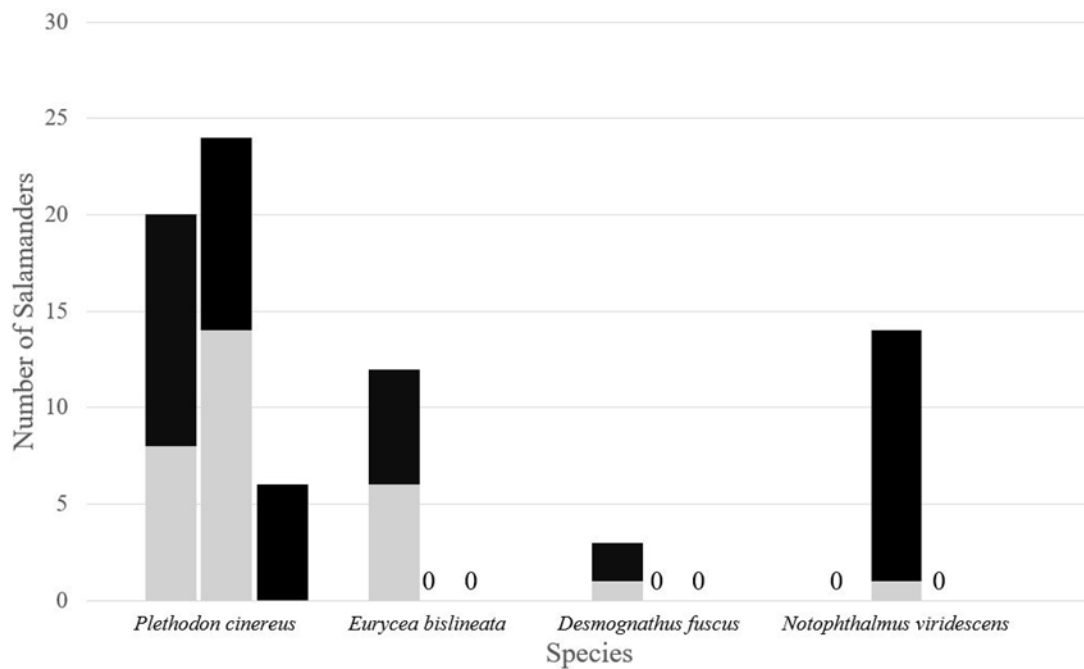
*Note:* Template for all locations except Lick Run. We established 20 meters along a trail with five tape measures at 5-meter increments perpendicular to trail and extended until 50 meters was reached to form 20m x 50m plot.

**FIG. 2.** Abundance of salamanders at different distances from hiking trails in central Pennsylvania.



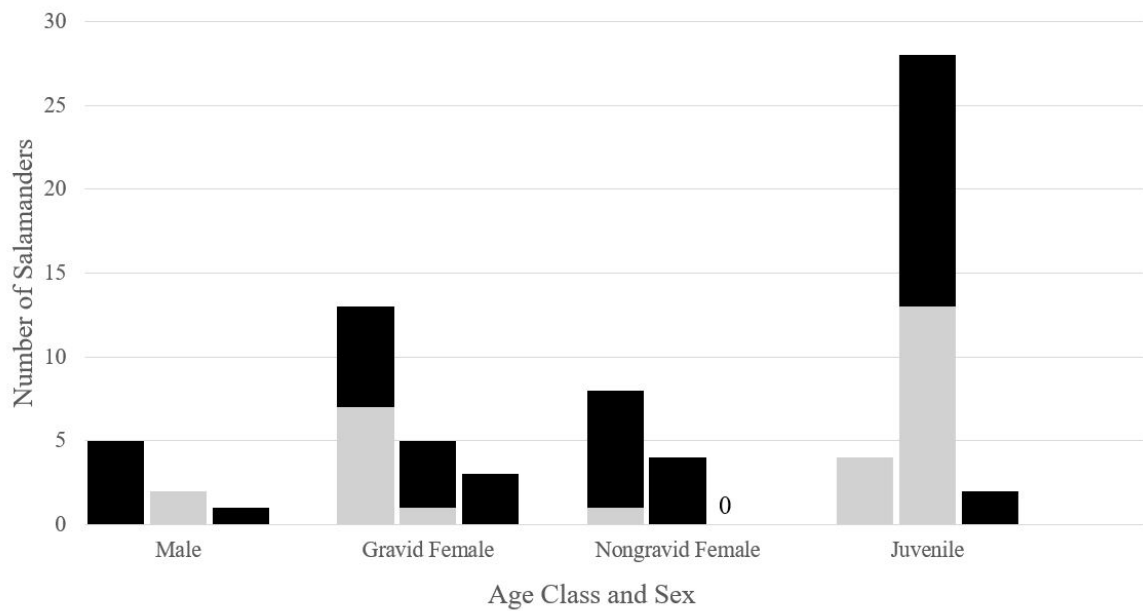
*Note:* Black bars represent the fall collection season and light gray bars represent spring collection seasons. The first bars represent collections at McElhattan Run, PA, the second bars represent collections Gottshall Run, PA, and the third bars if present represent collections at Lick Run, PA. There are no values for Lick Run above 30 meters from the trail (see methods section for description).

**FIG. 3.** Species variation of salamanders near hiking trails in central Pennsylvania.



*Note:* Black bars represent the fall collection season and light gray bars represent the spring collection season. The first bars represent collections at McElhattan Run, PA, the second bars represent collections Gottshall Run, PA, and the third bars if present represent collections at Lick Run, PA.

**FIG. 4.** Sex and age class of salamanders near hiking trails in central Pennsylvania.



*Note:* Black bars represent fall collection season and light gray bars represent spring collection season. The first bars represent collections at McElhattan Run, PA, the second bars represent collections Gottshall Run, PA, and the third bars, if present, represent collections at Lick Run, PA.

## Endnotes

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