

# The Importance of Microhabitat: A Comparison of Two Microendemic Species of *Plethodon* to the Widespread *P. cinereus*

Vincent R. Farallo<sup>1</sup> and Donald B. Miles<sup>1</sup>

**Understanding the role of microhabitats in the ecology of plethodontid salamanders is of utmost importance in the light of recent climate change. Plethodontid species are inherently susceptible to rising temperatures and drier conditions as they utilize cutaneous respiration. Furthermore, many species of plethodontid salamanders have restricted ranges, including species limited to single mountain tops, increasing the consequences of environmental change as their ability to disperse is limited. In this study we compare microhabitat data for a broadly distributed salamander species, *Plethodon cinereus*, and two microendemic species *P. sherando* and *P. hubrichti*. Our analyses evaluate two hypotheses. First, each of these species occupies microhabitat that differs from the available habitat. Second, microhabitat selection of the two microendemic species diverges from the widespread *P. cinereus*. In addition to testing these hypotheses, we provide additional data to highlight the importance of quantifying thermal microhabitats at different scales. Both *P. cinereus* and *P. sherando* were found in microhabitats that differed from randomly selected microhabitats. Moreover, *P. cinereus* occurred in habitats with high relative humidity and cooler air temperatures, whereas *P. sherando* occurred in habitats with warmer air temperatures but cooler substrate temperatures. These results suggest that habitat selection may play a role in the persistence of the range of *P. sherando* in contact zones with *P. cinereus*. Our data suggest that there may be habitat use differences between *P. cinereus* and *P. hubrichti*, but a limited sample size prevents us from making any firm conclusions. We also demonstrated variation in temperatures available in different microhabitats, which highlights the need to better understand microhabitat use as well as how these microhabitats will be affected by climate change.**

A major question in ecology concerns the factors that determine the distribution of species (Brown, 1984; Rosenzweig, 1995; Werner et al., 2014). Abiotic and biotic factors may jointly interact to generate a mosaic of environments that structure the potential distribution of a species. For many species (particularly in ectotherms), climate and abiotic conditions may be the primary drivers of a species distribution (Davis and Shaw, 2001; McCarty, 2001; Walther et al., 2002). Characterizing a species distribution has taken on new urgency as ecologists and conservation biologists struggle to predict biotic responses to climate change.

Most analyses of species distributions focus on a niche-theoretical framework to elucidate the factors constraining habitat occupancy of a species (Werner et al., 2014). However, this approach emphasizes habitat characteristics in coarse detail in order to use modern statistical methods to predict a species range using occurrence data. Species distributional models that use species presence data in conjunction with an ensemble of environmental data have been used to predict species responses to changing climates (Fitzpatrick et al., 2013). Recent methods have incorporated physiological and energetic data to refine (Kearney and Porter, 2004, 2009) and predict species ranges (e.g., Buckley et al., 2010).

Macroscale models of species distributions may provide the ability to forecast the distribution of widespread species. However, modeling the niche characteristics of microendemic species requires abiotic and biotic data at a finer resolution. A first step entails quantifying those factors affecting the performance of a species. Moreover, the selection of variables should include environmental aspects that are likely to affect a species population growth rate. In ectothermic organisms, linking habitat variation to population growth rates can be accomplished by modeling how the thermal environment affects physiological performance.

Because performance is known to influence key components of fitness (e.g., growth, survivorship, and reproduction), quantifying those environmental attributes that may determine the ability of an individual to attain a physiologically active temperature is a first step. Huey (1991) presented a schema illustrating how the abiotic environment may impinge on an organism's fitness via  $T_b$ . We have modified the schema to include cutaneous water loss, combined with  $T_b$ , as "filters" which can impact an organism's fitness. Jointly, these two variables may affect physiological capacities, such as locomotor performance (e.g., Preest and Pough, 1989; Titon et al., 2010). Individual variation in physiological performance has fitness consequences by affecting growth rates (Sinervo, 1990), survivorship (Miles, 2004), mating opportunities (Robson and Miles, 2000), and fecundity (Stahlschmidt et al., 2013).

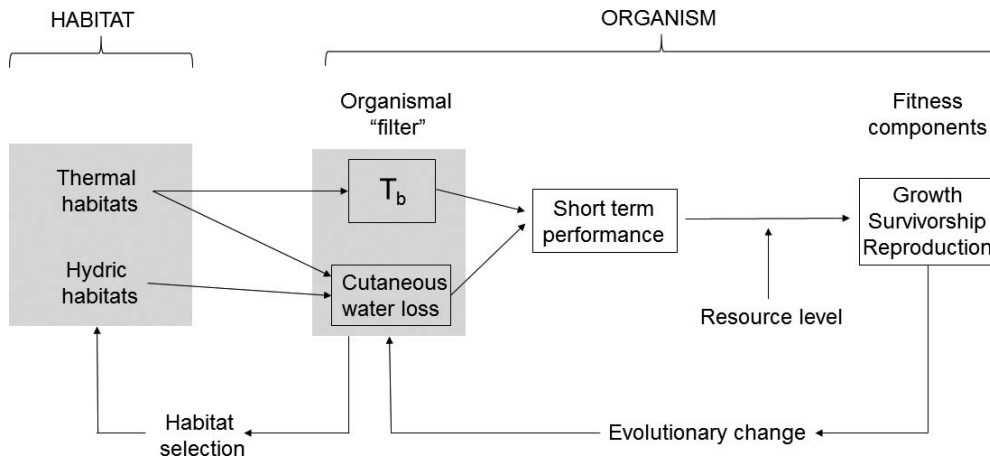
***Plethodontid salamanders as a model system.***—Plethodontid salamanders are one of the most abundant vertebrates in forests of the eastern United States (Semlitsch et al., 2014). This group of salamanders has a critical role in regulating invertebrate detritivores (Walton, 2005, 2013; Walton et al., 2006). They also function as energy capacitors through storage of nutrients in forest ecosystems (Hickerson et al., 2012; Semlitsch et al., 2014). Thus, plethodontid salamanders serve as a key indicators of forest health because of their sensitivity to habitat disturbances and ease of sampling (Welsh and Droege, 2001).

The habitat requirements for plethodontid salamanders are structured by several physiological constraints as illustrated by Huey (1991; Fig. 1). First, a primary physiological feature of *Plethodon* salamanders is their reliance on cutaneous respiration. Consequently, individuals require cool and moist habitat to persist (Spotila, 1972; Gatz et al., 1975; Wells, 2007). The second attribute of *Plethodon* salamanders is their nocturnal activity period, which limits their chance of

<sup>1</sup> Ohio University, Department of Biological Sciences, 107 Irvine Hall, Athens, Ohio 45701; Email: (VRF) vfarallo@gmail.com. Send reprint requests to VRF.

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**Fig. 1.** The distribution of a plethodontid salamander species is affected by the habitat characteristics, such as ambient temperature and hydric environments that optimize short-term physiological performance. Variation in these two categories can affect population dynamics via their influence on key fitness traits, such as growth, survivorship, and reproduction. Modified from Huey (1991).

overheating, but also means that any active thermoregulation they utilize must involve exploiting microhabitats which have been heated at different rates during the day. Most plethodontids are imprecise thermoregulators (Feder, 1982), but there is evidence that species may select temperatures through habitat selection. Broadly, *Plethodon* salamanders can move vertically in the soil through the use of burrows and natural crevices created by rocky substrates to find appropriate moisture and temperature (Diefenbacher, 2007; Caceres-Charneco and Ransom, 2010; Drake et al., 2012). This vertical movement allows them to persist when surface conditions are not ideal, including using below ground refugia during cold winter months (Grizzell, 1949; Caldwell and Jones, 1973; Caldwell, 1975; Hoff, 1977) as well as when surface conditions are too dry or warm during the rest of the year (Stebbins, 1954; Houck, 1977; Camp, 1988).

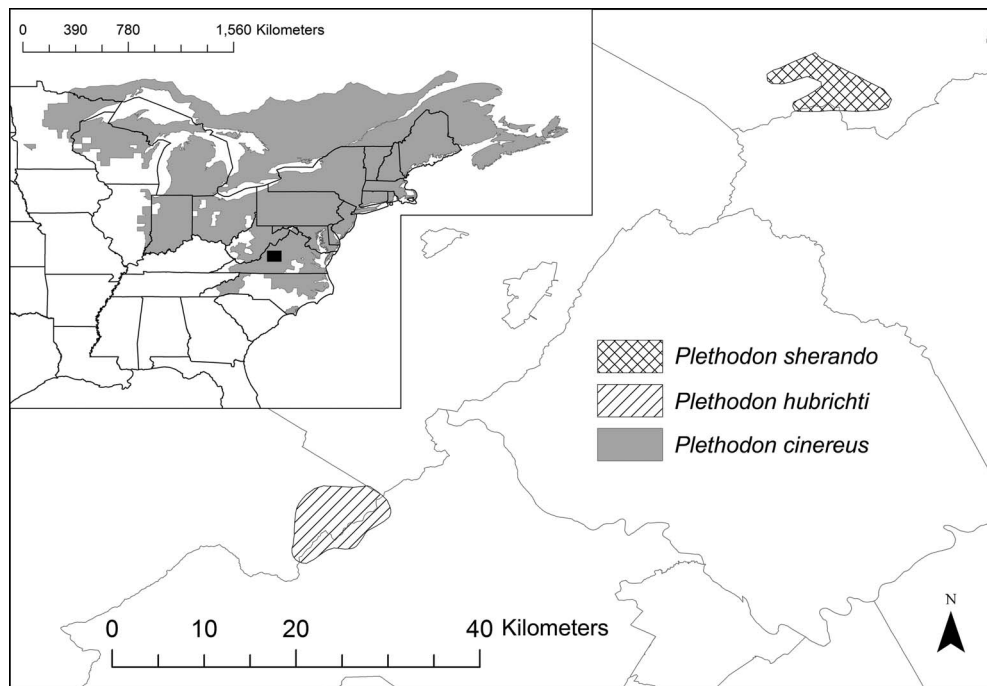
The requirement for thermally appropriate microhabitats is especially important in light of the planet's changing climate. There is predicted to be substantial loss of suitable habitat as a result of climate change (Milanovich et al., 2010). Salamanders are especially vulnerable to habitat changes. Their physiological requirements for cool and moist habitat may drive dispersal to higher elevations until suitable habitat is no longer available. Therefore, climate change is expected to have detrimental impacts on these high elevation species (Raxworthy et al., 2008; Xu et al., 2009). Species range shifts have already occurred as a result of climate change (Walther et al., 2002; Parmesan, 2006). Some salamander species have shown a decline in body size, presumably in response to climate change (Caruso et al., 2014), which could impact the habitat they are able to utilize or cause changes in interspecific competition. Additionally, similar to the potential impacts of climate change, other disturbances that alter the forest floor habitat have been shown to be detrimental to salamander populations (Gibbs, 1998; Hocking et al., 2013).

One advantage that amphibians, as well as other small ectotherms, have is that they are capable of utilizing microhabitat refugia, which may help mitigate the effects of climate change (Seebacher and Alford, 2002; Shoo et al., 2011). In addition to temperature, amphibians also need to regulate moisture, making microhabitats especially critical for maintaining physiological performance (Spotila, 1972). Microhabitat refugia include leaf litter and cover objects, such as rocks, woody debris, or other substrates that buffer against warm, dry, or both habitats (Jaeger, 1972; Grover, 1998; McKenny et al., 2006; Patrick et al., 2006). Time since last rain has been shown to improve the quality of microhabitats (e.g., increasing moisture on the forest floor

and increase surface activity of *P. serratus* (O'Donnell et al., 2014). Quantifying how microhabitats are affected by regional scale climate variables as well as the conditions in which salamanders exploit microhabitat refugia will facilitate predictions regarding potential changes to species distribution or ecology.

Plethodontid salamanders are characterized by having small home ranges; however, individuals within a population may utilize a diverse array of microhabitats within a small area, including arboreal substrates (Jaeger, 1978; Trauth et al., 2000; Regester and Samoray, 2002; Niemiller, 2005), various types of cover objects (Jaeger et al., 1982; Mathis, 1990), and burrows below the forest floor (Diefenbacher, 2007; Caceres-Charneco and Ransom, 2010; Drake et al., 2012). Therefore, determining detectability is important when attempting to assess the status of populations which will be further enhanced by increasing our knowledge of microhabitat use (Bailey et al., 2004a, 2004b, 2004c; Dodd and Dorazio, 2004; MacKenzie et al., 2004). Previous studies have assessed the habitat associations of widespread plethodontids (Gibbs, 1998; Hocking et al., 2013; Peterman et al., 2013). However, we have scant information regarding specific ecological requirements of many microendemic salamander species. The absence of key data on habitat requirements complicates our ability to assess population status and predict the consequences of anthropogenic disturbance.

The primary goal of this study is to assess the microhabitat use of two microendemic species whose natural history is largely unknown: the Peaks of Otter Salamander (*P. hubrichti*) and the Big Levels Salamander (*P. sherando*). We use these data to compare with the closely related and widespread congener, the Eastern Red-backed Salamander (*P. cinereus*). The distribution of *P. cinereus* spans over 1.8 million km<sup>2</sup> (Fig. 2) and completely surrounds the range of both microendemic species, but includes only a narrow area of sympatry with both species (Highton, 2004; Kniowski and Reichenbach, 2009). *Plethodon hubrichti* is hypothesized to be the sister taxon to *P. nettingi* (Highton et al., 2012), another endemic *Plethodon*. However, *P. hubrichti* is also hypothesized to be more closely related to *P. cinereus* than *P. sherando*, whereas *P. sherando* is the sister taxon to *P. serratus*, the Southern Red-backed Salamander (Bayer et al., 2012). Interestingly, the range of *P. sherando* is completely surrounded by *P. cinereus*, while *P. serratus* is found over 370 km from *P. sherando*. Both microendemic species have restricted ranges of ~36 km<sup>2</sup> and ~49 km<sup>2</sup> for *P. sherando* and *P. hubrichti*, respectively (Fig. 2). The restricted ranges of these species and their high elevation habitats make them vulnerable to



**Fig. 2.** Distributions of *Plethodon cinereus*, *P. sherando*, and *P. hubrichti* taken from IUCN Redlist ([www.iucnredlist.org](http://www.iucnredlist.org)). The inset map in the top left corner contains the range of *P. cinereus* and also denotes with a black rectangle the extent of the main map within the US.

population declines from climatic warming. Warmer and drier environments at low elevations will force most movement toward even higher elevation habitats. Furthermore, both of their ranges are encompassed by *P. cinereus*, which means any expansion or change in the geographic distribution of the microendemics as a consequence of climate change may create a challenge for population persistence through competition. For example, *P. cinereus* is thought to have contributed to the decline of the Cheat Mountain Salamander (*P. nettingi*) in the past 30 years as a result of the former species exhibiting an upward shift in elevation and increasing in abundance (Kroschel et al., 2014). Character displacement also occurs in areas where another species, *P. hoffmani*, is sympatric with *P. cinereus*, indicating that competition occurs between small bodied *Plethodon* (Jaeger et al., 2002). Despite the potential threats to both species, little is known about either species' ecology. Kniewski and Reichenbach (2009) conducted a general assessment of sympatric populations of *P. hubrichti* and *P. cinereus*, while Mitchell et al. (1996) and Reichenbach and Sattler (2007) showed that clear cutting of forests had a negative effect on populations of *P. hubrichti*. To our knowledge, no studies have addressed the ecology of *P. sherando*. Furthermore, no studies have assessed detailed habitat use, such as soil moisture, relative humidity, and temperature of microhabitats for either of the microendemic species. In order to assess the potential impacts of climate change on species with highly restricted ranges, we need a better understanding of how species utilize habitat at a biologically realistic scale.

We test two hypotheses in this study. First, do the three species exhibit a preference for habitat characteristics that differs from the available habitat? Second, do the microhabitats used by the microendemic species overlap with the preference exhibited by the widespread species, *P. cinereus*? We predict that the widespread species, *P. cinereus*, should exhibit less selectivity than either of the microendemics. This prediction is based on the premise that *P. cinereus* is expected to be a habitat generalist that exploits multiple types of habitats. Furthermore, the microendemics are predicted to

have specialized habitat preferences and exhibit limited overlap with that of *P. cinereus*. We also comment on the role of microhabitats as potential refugia from climate change. Finally, we suggest additional avenues of research that would enhance our knowledge of these microendemic plethodontids and enhance predictions regarding their vulnerability to climate change.

## MATERIALS AND METHODS

**Field sites.**—We measured microhabitat variables at 39 localities throughout the Appalachian Mountains and foothills within the range of *Plethodon cinereus* (35), *P. sherando* (2), and *P. hubrichti* (2) between 8 June 2012 and 18 October 2014. Each locality consisted of an area of 1 km<sup>2</sup>. At each locality we searched sites for salamanders using time constraint surveys of 1 person hour each and haphazard searching. Surveys were conducted during the day when salamanders are under cover and in the evening when they are active. We completed a total of 99 surveys, with each locality being surveyed between 1–5 times. Field sites for *Plethodon cinereus* were located between 39.6° and 36.0° latitude and represented the southern half of the species range. This latitudinal band is comparable with the distribution of the two microendemic species from Virginia.

**Microhabitat measurements.**—We collected microhabitat data at the capture location of each salamander. We also obtained habitat data at ten randomly chosen points within the search area. The random points were selected to provide information on the availability of each microhabitat category included in our study. All random points were found by using a random number generator to determine a compass bearing and then walking ten meters in that direction. In addition to the systematic collection of random points, we also included points ten meters in a random direction from presence points and also under cover objects within 1 m<sup>2</sup> areas around presence points where salamanders were found. Each random absence point represents a location that did not have a salamander present during our survey. We do not



mean to imply that no salamander ever occurs at our absence points, but rather that a salamander is not present and surface active under the current microhabitat conditions. These sampling methods ensured that we located salamanders in as many surface active microhabitat locations as possible as well as thoroughly sampling microhabitats available for them to potentially utilize. During surveys we collected ecologically relevant environmental data for plethodontid salamanders that rely on cutaneous respiration. Air temperature ( $\pm 0.5^\circ\text{C}$ ) and relative humidity ( $\pm 3\%$ ) were both recorded 1 m from the ground using a Kestral 3500 weather meter and digital psychrometer. We used measurements at 1 m because these are comparable to broad scale climatic data (e.g., WorldClim). We recorded soil temperature using either a ThermaPlus thermocouple meter or an infrared thermometer (IRT) with a high sensitivity probe ( $\pm 0.5^\circ\text{C}$ ; Thermoworks Inc.). We measured the temperature of the ground and substrate surface with an infrared thermometer ( $\pm 0.6^\circ\text{C}$ ; Thermoworks Inc.). We measured soil moisture using a HydroSense II ( $\pm 3\%$ ; Campbell Scientific Inc.). Soil moisture and temperature probes were all inserted at approximately a  $45^\circ$  angle. Our microhabitat sample consisted of 152 presence and 839 absence points (991 total).

We also placed iButton temperature loggers (Model: DS1922L, Embedded Data Systems) at various microhabitats at a site in Perry County, Ohio within habitat of *P. cinereus*. The loggers were placed in five locations, 50 cm above ground, ground level, and 10, 20, and 30 cm below ground level from 14 July through 24 August 2012. We used these data to quantify the temporal change in the thermal environment.

**Statistical analyses.**—We classified each site into one of eight categories. The first three categories were the capture locations for each of the focal species, another three categories included the random points within the range of each of the focal species, and the final two categories consisted of random points within the range of *P. cinereus* but located within 10 km of the range of *P. sherando* or *P. hubrichti*.

In order to visualize differences in habitat among these categories, we utilized non-metric multidimensional scaling and plotted the 95% confidence ellipses. We used a Generalized Linear Mixed Models (GLMM) from the R package lme4 (Bates et al., 2014) with site treated as random factor and the habitat characteristics as predictor variables to determine if each species utilized the microhabitat variables different from what was available. We also compared the parameter estimates to infer whether each species used similar habitat variables. Prior to running the GLMM, we screened the data for evidence of multicollinearity by examining the pairwise correlations among all variables. None of the correlations exceeded 0.70, suggesting multicollinearity is not likely to affect the results of the GLMM. We used the function “dredge” from the package MuMIn (Bartoń, 2013) to compare all possible subset models out of the possible models using a saturated model as the initial model. The “dredge” function uses AICc scores to select the best reduced model from the original saturated model. The marginal  $R^2_{\text{GLMM}}$  was also calculated for each model using a modified method of Nakagawa and Schielzeth (2013; see Johnson, 2014) using the best supported model for each species. If there are significant differences in habitat use for specific microhabitat variables at presence sites compared to absence sites, the marginal  $R^2$  provides an indication of the

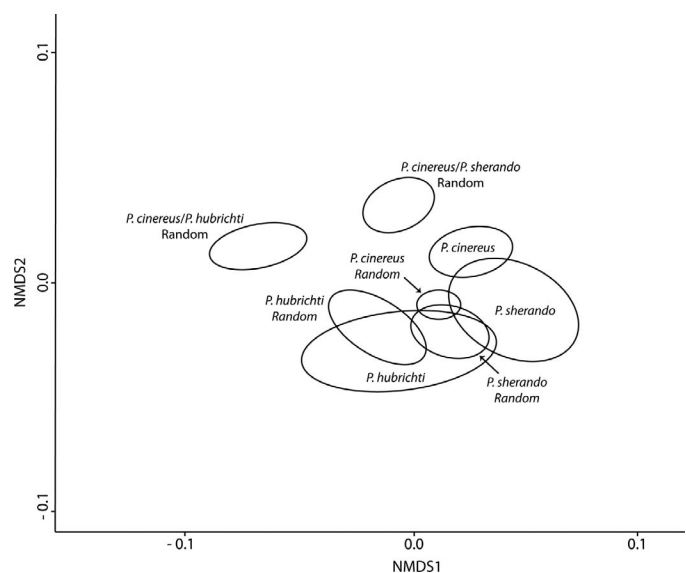
**Table 1.** The correlation of environmental variables to the two NMDS axes.

	NMDS 1	NMDS 2
Air temperature	0.328	-0.556
Soil temperature	0.546	0.336
Ground temperature	0.263	-0.571
Soil moisture	-0.926	0.045
Relative humidity	0.270	0.752

microhabitat variables selected by each species. Comparison of significant microhabitat variables for each species assists in determining the potential mechanisms for the absence of overlap between these microendemic species and the widespread *P. cinereus*. All statistical analyses were conducted using the program R (ver. 2.15; R Core Team, 2012).

## RESULTS

**Variation in microhabitat traits.**—Analysis of the microhabitat characteristics by the NMDS revealed substantial variation across all groups. The first axis described a soil temperature and soil moisture gradient. Points on the positive pole consisted of sites with high soil temperatures and low soil moisture (Table 1); whereas, points positioned on the positive end of the second axis have high above ground relative humidity and points towards the negative pole had high values for air temperature (1 m above ground) and below ground soil temperatures (Table 1). The first axis separated *P. sherando* from *P. cinereus* and *P. hubrichti* (Fig. 3), which suggests the former species tolerates warmer and drier soil surfaces. The second axis positioned *P. hubrichti* and *P.*



**Fig. 3.** Results from a non-metric multidimensional scaling analysis of microhabitat data. The 95% confidence ellipses are presented for the capture points for *Plethodon cinereus*, *P. sherando*, and *P. hubrichti* (3), random points within the sample plot for each species (3), and random points that are within 10 km of the range of *P. sherando* and *P. hubrichti* (2). The first axis describes soil temperature and soil moisture. Ellipses on the positive pole consisted of sites with high soil temperatures and low soil moisture. The second axis describes relative humidity as well as air and soil temperature. Ellipses positioned on the positive end of the second axis have high relative humidity, and ellipses towards the negative pole had high values for air temperature (1 m above ground) and below ground soil temperatures.

**Table 2.** The results of Generalized Linear Mixed Models comparing the habitat variables that best predict the presence sites of *Plethodon cinereus* and *P. sherando*. Parameter estimates and their standard error are presented. Values in bold indicates  $P < 0.05$ ; – indicates that a variable was not included in the best model for that species.

	Species			
	<i>Plethodon cinereus</i>		<i>Plethodon sherando</i>	
	Parameter estimate	Standard error	Parameter estimate	Standard error
Air temperature	0.067	0.046	<b>1.904</b>	<b>2.255</b>
Soil temperature	–	–	<b>–0.761</b>	<b>0.165</b>
Ground temperature	<b>–0.381</b>	<b>0.059</b>	<b>–0.968</b>	<b>0.206</b>
Soil moisture	–	–	–	–
Relative humidity	<b>–0.047</b>	<b>0.008</b>	–	–

*sherando* at the negative part of the habitat gradient and *P. cinereus* in the positive zone. Thus, *P. cinereus* occupied sites with higher relative humidity and lower temperatures. Both *P. hubrichti* and *P. sherando* occurred in sites with warmer temperatures. Examination of the 95% confidence ellipses reveals that there is some overlap in the microhabitat variables that characterize the capture sites for the three salamanders. An analysis of distance (function ADONIS) found significant differences between *P. cinereus* and *P. sherando* ( $F_{1,136} = 303.74$ ,  $P = 0.024$ ) but no difference between *P. cinereus* and *P. hubrichti* ( $F_{1,111} = -8.274$ ,  $P = 0.75$ ).

The widespread *P. cinereus* showed no overlap between the ellipses for the capture and random points. In contrast, both microendemic species exhibited variable amounts of overlap between the capture and random points. The ellipses for *P. hubrichti* almost completely overlapped, although the orientation of the random and capture ellipses differed. The random points for *P. sherando* overlapped with the capture points but not as extensively as *P. hubrichti*. Note that the size of the confidence ellipses for *P. cinereus* are smaller than either microendemic species. There is also a striking concordance between the confidence ellipses for *P. cinereus* and *P. sherando*. This suggests that although the species occupy environments with similar habitat structure, they are selecting divergent microhabitat features. Finally, the two categories that included random points at sites within the range of *P. cinereus* but directly adjacent to random points of *P. sherando* and *P. hubrichti* were positioned away from both the capture points for the three salamander species and their associated random points.

**GLMM analysis.**—We applied a generalized linear mixed model to determine which habitat variables predict the presence of each species. Because of the limited number of capture points for *P. hubrichti*, we did not include the species in the GLMM analysis. However, we present the patterns for habitat use as a comparison with the remaining species. We detected significant differences between the habitat characteristics of capture sites and random points for both *P. cinereus* and *P. sherando* (Table 2). The best model for *P. cinereus* (marginal  $R^2_{\text{GLMM}} = 76\%$ ) included two significant variables (ground temperature and relative humidity). The best model for *P. sherando* (marginal  $R^2_{\text{GLMM}} = 41\%$ ) involved three variables (air temperature, soil temperature, and ground temperature). *Plethodon cinereus* was found at significantly cooler ground temperature and lower relative humidity than what was available (Fig. 4). *Plethodon sherando* was found at areas with significantly higher air temperature and lower ground and soil temperatures (Fig. 4). Although the GLMM found no difference in soil moisture used by any of the

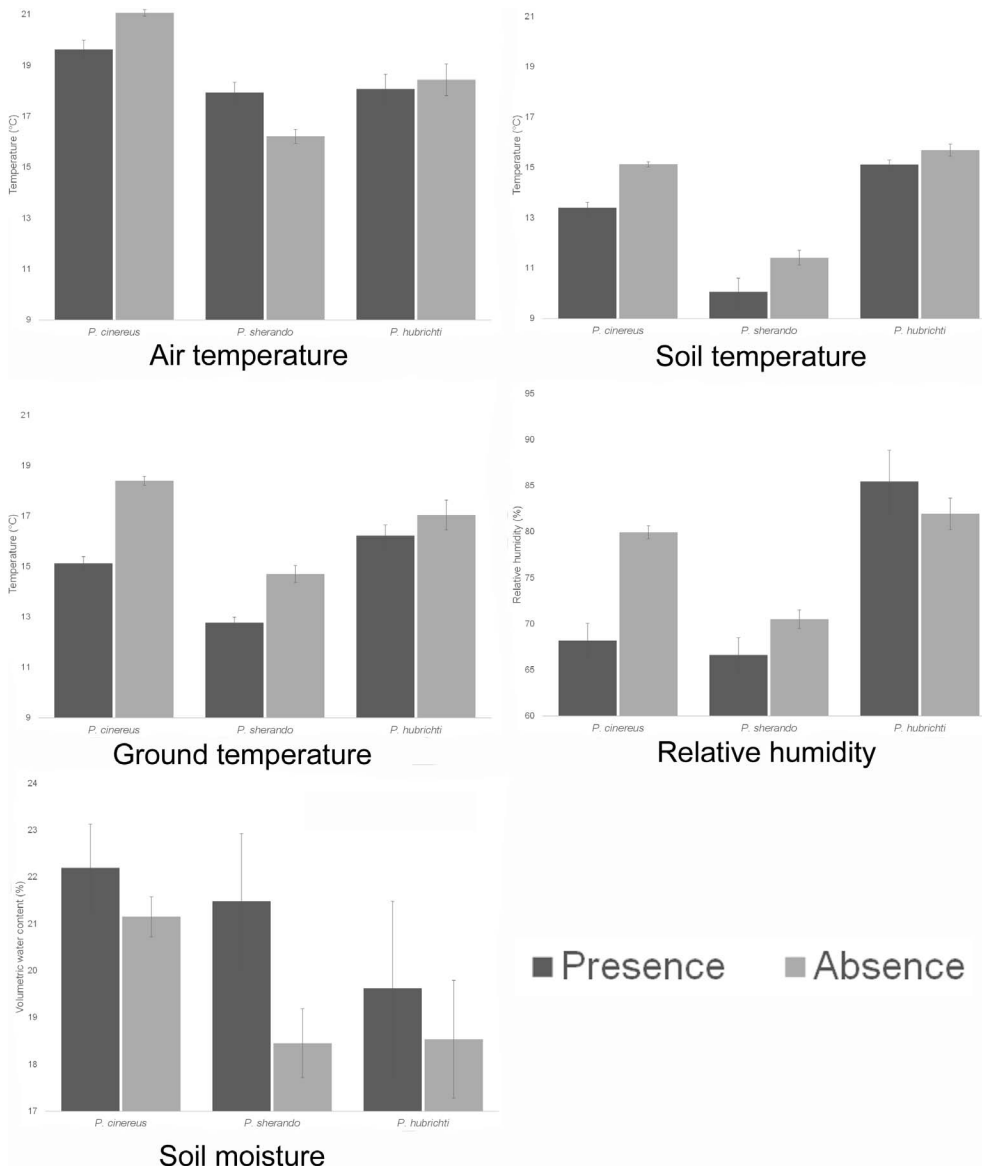
species compared to what was available, *P. sherando* was found in areas on average with 2% higher soil moisture.

**Temporal change of microhabitats.**—The thermal characteristics of below ground microhabitats exhibited remarkable consistency in both mean values throughout the day (Fig. 5A) and over the entire period of deployment (Fig. 5B). Moreover, the logger positioned 50 cm above ground had a temperature range of 9.6°C between the hourly minimum and maximum temperatures over the course of an average 24-hour period, which was the highest value found among all sites (Fig. 5A). Notably, the logger 30 cm below ground only spanned 0.2°C on average over a 24-hour period (Fig. 5A). When considering the temperatures experienced throughout the entire deployment, the logger 10 cm below ground only varied 8.5°C with the logger at 30 cm below ground staying within 5.0°C throughout the 41 days (Fig. 5B). In contrast, the logger at ground level spanned 25.0°C, and the logger at 50 cm above ground had the greatest temperature range of 29.6°C (Fig. 5B).

## DISCUSSION

We found *Plethodon cinereus* and *P. sherando* utilize significantly different microhabitats than what is available, indicating that, at least when they are at the surface, they are seeking out specific habitats. In contrast, *P. hubrichti* showed substantial overlap between the habitat characteristics at the site of capture and the random points. All three species exhibited variable amounts of overlap with each other in the microhabitat space. Furthermore, different microhabitat variables seemed to be important for each species. Although we could not include *P. hubrichti* in a GLMM analysis, the species does not appear to exploit habitats that differ from the available habitat characteristics. *Plethodon hubrichti* also appears to utilize different habitat than *P. cinereus* based on our NMDS results; however, our statistical analysis did not support this conclusion. Given the low number of capture points for *P. hubrichti*, these results are tentative and require additional sampling while potentially including variables not included in this study, such as vegetation or soil characteristics.

The distribution of *P. sherando* includes only a small area of sympatry with *P. cinereus* (Highton, 2004). Our results suggest that the species are utilizing different microhabitats. One potential explanation for this pattern is that competition is structuring the habitat differences. Many species of *Plethodon* exhibit territoriality, often defending cover objects from other conspecifics (Jaeger and Forester, 1993; Mathis et al., 1995). However, some species exhibit territorial behaviors

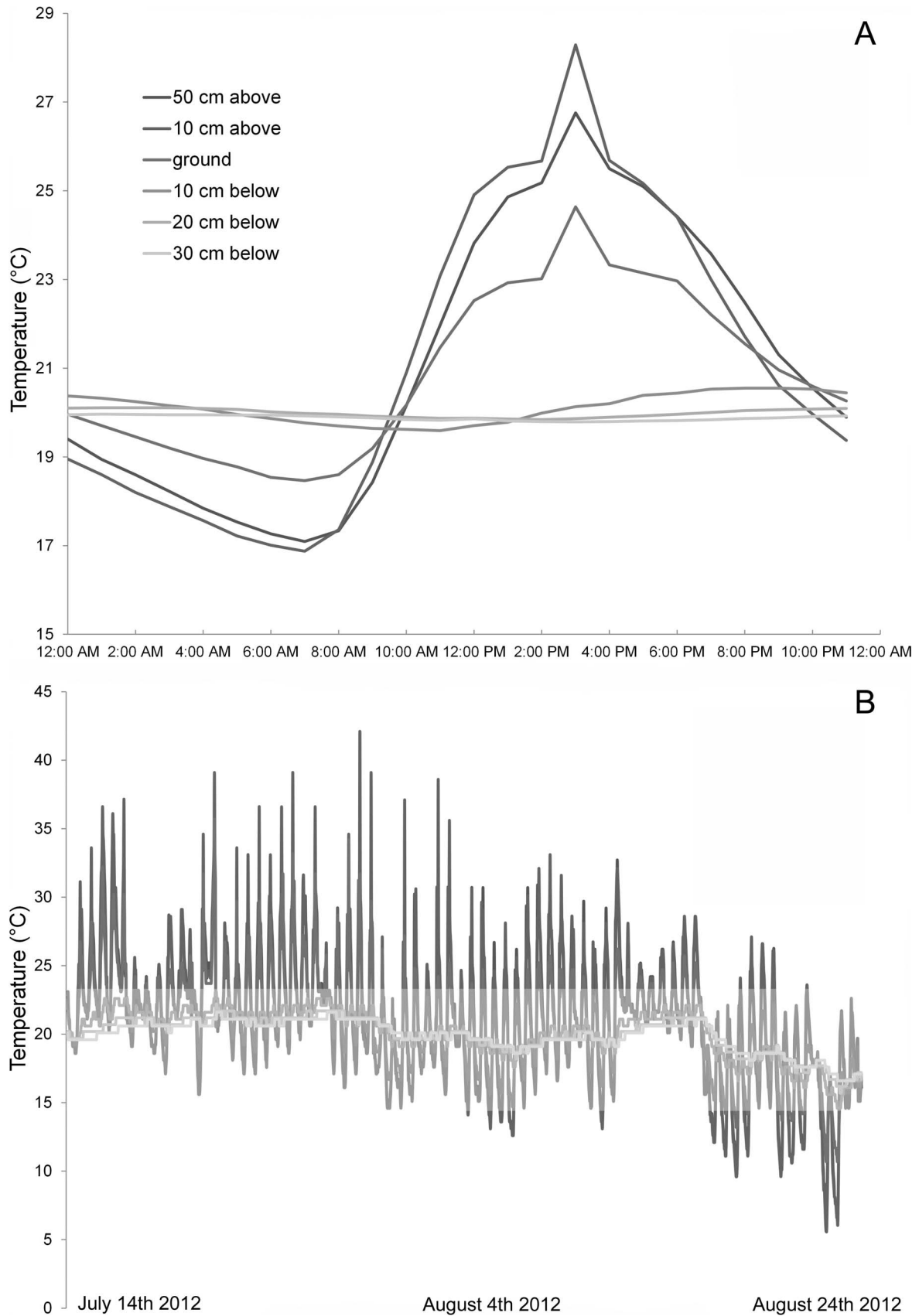


**Fig. 4.** Comparison of microhabitat variables for *Plethodon cinereus*, *P. sherando*, and *P. hubrichti* at capture sites (dark gray) and absence points (light gray). Values are means  $\pm$  standard error.

towards heterospecifics which can result in one species excluding the other from specific habitats (Jaeger, 1971; Anthony et al., 1997; Griffis and Jaeger, 1998). Furthermore, Jaeger et al. (2002) showed character displacement occurs in areas of sympatry between *P. cinereus* and *P. hoffmani*. In addition, the GLMM analyses revealed that the habitat traits predicting the occurrence of both species differed. This heterogeneity in microhabitat selection is also consistent with morphological differences between the species. The microendemic species *P. sherando* is morphologically distinct, having a larger head and longer limbs (Highton, 2004). These morphological features may enhance the ability of *P. sherando* to access thermally and hydrically favorable microhabitats. Within the small range of *P. sherando*, there is a high density of large rocky substrates that extend below ground level (Farallo, pers. obs.). We have observed areas where *P. cinereus* are found in soil dominated habitat, but in an area immediately adjacent where rocky habitat is dominant, *P. sherando* have been found (Farallo, pers. obs.). The use of rocky substrates has been associated with long limbs in Australian skinks (Goodman et al., 2008). Longer limbs may allow *P. sherando* better access to preferred microhabitats available in open pockets within rocky crevices below the

forest floor. However, limb length may entail a trade-off by limiting access to preferred thermal microhabitats that are dominated by soil and finer substrates. Davis and Pauly (2011) observed larger heads in a group of Western Slimy Salamanders (*P. albagula*) that routinely use subterranean karst habitat when compared to populations that are more likely to utilize terrestrial habitat. *Plethodon sherando* has a larger head than *P. cinereus* which may have an unforeseen benefit when utilizing these rocky underground habitats. There may also be morphological traits that are plastic, such as vertebrae number resulting in variable levels of elongation, allowing populations to better exploit different habitat conditions (Jockusch, 1997). Additional studies to quantify the performance differences of *P. sherando* when using subterranean environments could determine the role of limbs in habitat selection. Furthermore, limited data are available for microhabitat temperatures and moisture levels in below ground refugia.

A key question in biogeography and conservation biology is what determines the heterogeneity in species distributions, especially microendemic species. One potential explanation for the limited range of *P. sherando* and *P. hubrichti* is that *P. cinereus* typically outcompetes other small-bodied species of



**Fig. 5.** Temporal fluctuation in temperature recorded at five different microhabitats in Perry County, Ohio. (A) Diurnal variation in mean at each microhabitat. (B) Seasonal variation in temperature between 14 July and 24 August 2012.



*Plethodon* (Jaeger, 1970, 1972; Adams and Rohlf, 2000; Jaeger et al., 2002; Kroschel et al., 2014). The ability of *P. cinereus* to thrive in a broad array of forest habitats results in other microendemic species being restricted to specific types of habitat where they are able to gain a competitive advantage. Conversely, in lab based behavioral trials, *P. hubrichti* is more aggressive than *P. cinereus* (Arif et al., 2007), but it appears that *P. hubrichti* is restricted by abiotic factors (Arif et al., 2007) which results in their small range despite being competitively superior to *P. cinereus*. However, these results do not take into account microhabitat use as well as behavioral interactions that may be affected by differences in microhabitat. The laboratory behavioral trials were performed under standardized conditions, and they only included climatic data as abiotic factors. These results certainly provide possibilities for the restricted range of *P. hubrichti* and their interactions with *P. cinereus*; however, understanding how *P. hubrichti* utilizes microhabitat and consequently how they interact with *P. cinereus* when in those specific microhabitats will most likely provide a more complete understanding of their restricted range.

Our results partially support this pattern of habitat differentiation. The position of the 95% confidence ellipses for both *P. sherando* and *P. hubrichti* are significantly shifted away from *P. cinereus*. However, comparisons of the microhabitat characteristics between capture and random points for *P. hubrichti* exhibited substantial overlap, suggesting the species is not selective, at least given our limited data. *Plethodon sherando* shows little overlap between occupied and random points. The species tends to favor warmer habitats that have cooler ground and soil temperatures. Interestingly, the microhabitat features of the random points for *P. cinereus* and *P. sherando* occupy similar sections of the microhabitat space defined by the NMDS analysis. In contrast, *P. sherando* inhabits forested environments that are similar to *P. cinereus* but has different thermal requirements. Our results emphasize the need to include behavioral and ecological traits to enhance our ability to determine how changes to habitat will affect species persistence.

The thermal data derived from the deployment of dataloggers provide a key pattern. We deployed the dataloggers during summer months when *P. cinereus* is not engaged in surface activity at low elevations. However, the thermal data demonstrate the consistency of below ground temperatures. Salamanders are able to seek refuge in microhabitats that provide a nearly constant temperature by venturing only 10 cm below ground. It is also very likely that a similar effect would be seen under leaf litter. Although this was not measured by our data loggers, we have seen striking differences in temperatures above and below leaf litter, including a 34.2°C difference at a field site in West Virginia where salamanders were present under leaf litter (54.2°C above leaf litter and 20.0°C below leaf litter; unpubl.). Another factor not measured by our data loggers is relative humidity within underground retreats which may provide pockets of higher humidity levels.

Our results revealed critical gaps in information that must be addressed in order to mitigate potential impacts of climate change on salamander populations. Given the numerous microendemic species whose distributions are limited to high elevation habitats, the potential for dispersal to thermally favorable microclimates is unlikely. Determination of the exploitation of below ground microhabitats by salamanders enhances our ability to design and implement new habitat studies. If salamanders are able to thrive with minimal or no

surface activity, including sufficient food acquisition and mating success, then habitat studies need to shift to include their below ground habitats. However, if the ability of salamanders to feed and mate requires surface activity, then research should focus on the impacts of climate change on these surface habitats even if they are only used for short periods of time.

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