Aspects of Eastern Wormsnake (*Carphophis amoenus*) Microhabitats at Two Natural Areas in Fairfax County, Virginia, and Anne Arundel County, Maryland

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Introduction

Carphophis amoenus (Say) (Eastern wormsnake; hereafter wormsnake) is a small, fossorial snake whose range encompasses most of the eastern United States, from southern Massachusetts to Georgia, and east to the Mississippi River (Powell et al., 2016). Ernst and Ernst (2003) estimate that wormsnakes are one of the most common vertebrates in eastern deciduous forests and thus may play an underappreciated role in forest food webs, particularly as a source of prey for other snakes (e.g. *Lampropeltis getula*, and *Coluber constrictor*; Clark 1970), and birds during the nesting season (e.g. *Siala sialis*; Stankback and Mercadante 2009). Despite their potential importance in eastern forest ecosystems, few studies have focused exclusively on this species and many questions remained unanswered about their ecology (Barbour et al. 1969, Russell and Hanlin 1999, Orr 2006).

One aspect of wormsnake ecology that remains poorly understood is their microhabitat requirements. The microhabitat encompasses the area needed for an individual to fulfill its immediate physiological needs, such as osmoregulation and thermoregulation (Huey 1991). Small snakes are especially sensitive to temperature changes and subcutaneous water loss, which render them more vulnerable than larger snakes to variable microclimates (Shoemaker and Nagy 1977, Stevenson 1985). Furthermore, optimal body temperatures for fossorial snakes may be shifted towards lower temperatures compared to other reptiles, requiring them to seek cool microclimates during hot weather (Kamel and Gatten 1983). Therefore a small, fossorial snake such as the wormsnake may require a microclimate that is both humid and cool for survival.

While wormsnakes spend approximately 60% of their time underground (Clark 1967), most of their time above ground is spent in an immobile resting state within refuges (Barbour et al. 1969, Ernst and Ernst 2003). Their most commonly used refuges are the interstitial spaces within coarse woody debris (CWD)—defined as fallen logs and branches with a diameter greater than 7.5 cm (Harmon et al. 1986). Some snakes use the microclimate found within CWD refuges to thermoregulate while simultaneously avoiding desiccation and predation (Elick and Sealander 1972, Huey et al. 1989, Winne et al. 2001). Hence, migration between the soil and CWD refuges may provide benefits for wormsnakes that have yet to be explored.

Previous investigations into the microhabitat preferences of wormsnakes used artificial refuges (e.g., plywood coverboards and concrete slabs) to attract snakes rather than relying solely on CWD (Russell and Hanlin 1999, Creque 2001, Orr 2006). Artificial refuges can have significantly greater thermal variation than CWD refuges and thus may alter the behavior of wormsnakes during the microhabitat selection process (Houze and Chandler 2002). Thus, previous studies using artificial refuges may not accurately reflect the microhabitat preferences of this species under natural conditions. Additionally, they have not examined why CWD appears to be a preferred refuge for this species in its native habitat. Furthermore, it is unknown whether other components of the habitat also play a role in microhabitat selection. For example, leaf litter and understory vegetation may provide wormsnakes cover from predators while moving above ground. Abiotic factors, such as soil moisture, may affect the ability of snakes to burrow or find earthworms, their primary prey (Barbour 1960). Hence habitat variables adjacent to refuges that could be important in the selection process may have been overlooked by previous studies.

The purpose of this study was to determine the microhabitat preferences of wormsnakes under natural conditions. Specifically, we attempted to determine (1) whether CWD that wormsnakes select as refuges have significantly different microclimates than CWD available within the habitat as a whole; (2) if components of the habitat immediately surrounding CWD refuges impact microhabitat selection; and (3) whether temperatures within CWD refuges fall within the species' thermal optima for a greater portion of the year than soil temperatures.

Methods

Study Sites: We sampled at Huntley Meadows Park (hereafter Huntley Meadows; 38°45'12.49" N, 77°06'25.64" W; Figure 1.) in Alexandria, Fairfax County, Virginia, and Jug Bay Wetlands

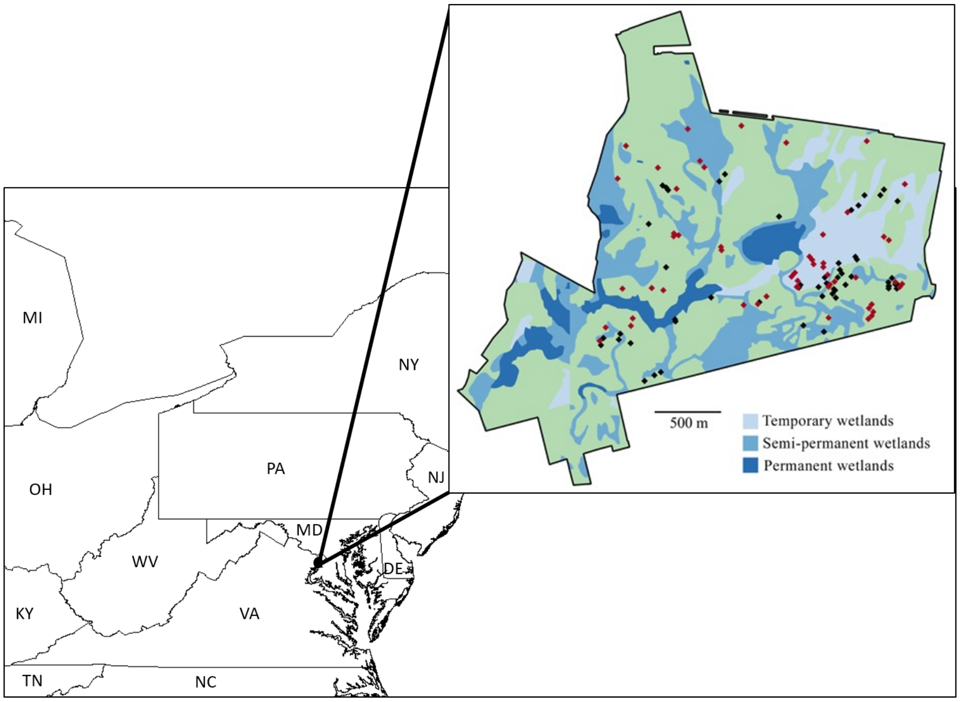


Figure 1. Map of Huntley Meadows Park. Snakes found in 2012 represented by red diamonds and in 2013 by black diamonds. Wetland classification based on US Fish and Wildlife Service's National Wetlands Inventory classification scheme.

Sanctuary (38°47’04.11’, 76°42’01.84” W; hereafter Jug Bay, Figure 2) in Lothian, Anne Arundel County, Maryland. Huntley Meadows is a 630 ha park containing a variety of habitats, including a 9 ha central wetland, meadows, vernal pools, and oak-maple forest. Huntley Meadows is surrounded by a highly developed landscape and has little connectivity with other non-urbanized habitats. At 650 ha, Jug Bay is similar in size to Huntley Meadows, but approximately half of that area is comprised of wetlands, leaving less terrestrial habitat appropriate for wormsnakes. Terrestrial habitats at Jug Bay consist of meadows, deciduous forest and mixed forest, bordered by agricultural fields and a river.

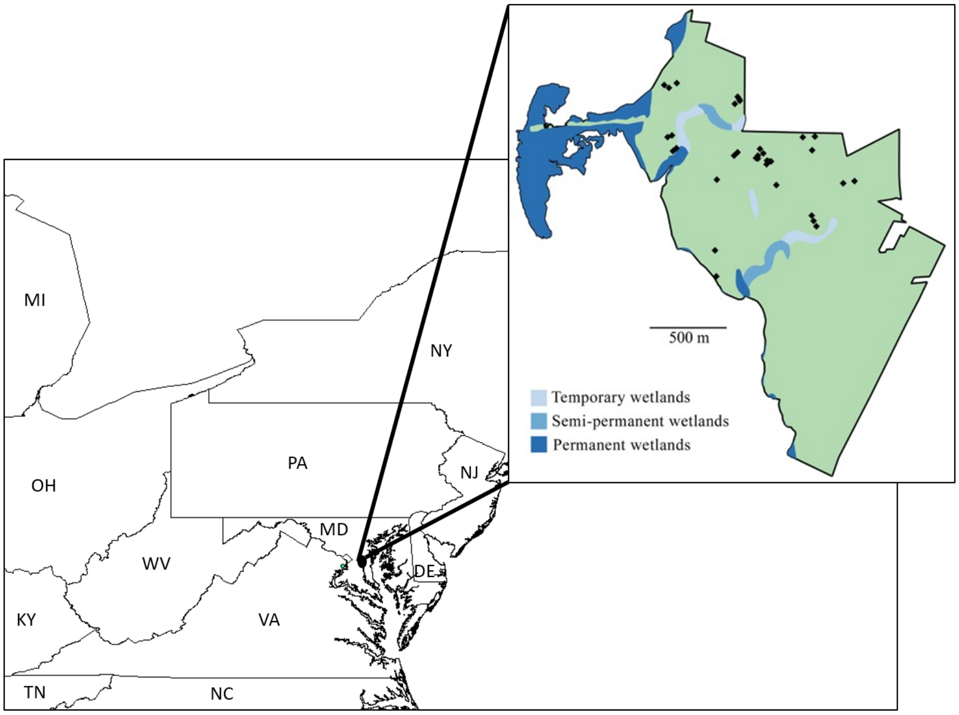


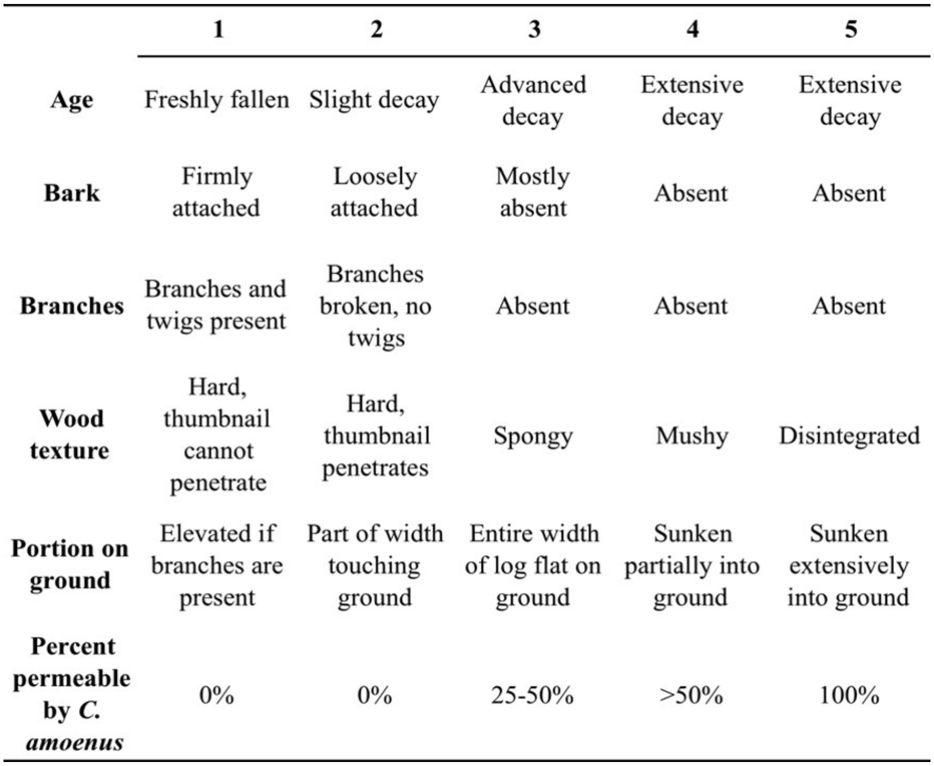
Figure 2. Map of Jug Bay Wetlands Sanctuary. Black diamonds represent snakes found in 2013. The southern portion of the park is mostly private farmland and was not included in searches.

Sampling Scheme: We used a stratified-random sampling procedure to ensure sampling was evenly distributed throughout available habitats. Using QGIS 2.0.1, we overlaid a vector grid (divided into 300 m x 300 m squares) on a shapefile of wetland coverage for each field-site (FWS 2014). If a grid square was dominated by unsuitable habitats (e.g. areas with saturated soils), it was discarded. We then used a random point function to choose an equal number of search points for each remaining square based on the proportion of existing available suitable habitat. Search points were a minimum of 100 m apart (see Barbour et al. 1969 for home range data) to avoid spatial autocorrelation and maintain observation independence by decreasing the likelihood of recapture (Koenig and Knops 1998).

Time Constrained Searches and Demographics: We conducted one-person-hour time-constrained searches at each preselected sampling point in 2013 and 2014 during May, June and July, when wormsnake detectability is highest (Orr 2006). We located sampling points in the field with a Garmin eTrex 20 GPS unit. Time-constrained searches consisted of meticulously looking beneath and within all available CWD within an 80 m radius of each randomly selected point. Once captured, we weighed snakes to the nearest 0.2 g using a Pesola® spring scale, and measured snout-vent and tail length with a Fisher® scientific measuring ruler. We sexed snakes using visual characteristics, tail length and thickness (*see* Fitch 1956). Individuals less than 170 mm in total length were considered juveniles (Willson and Dorcas 2004). We released snakes at the point of capture after measuring, weighing, and assessing body condition.

Microhabitat Measurements: We recorded a suite of microhabitat features, both at the capture point and within a 1 m^2 quadrat centered on that point. We measured refuge temperature and humidity with an Extech® EasyView 20 digital thermo-hygrometer and CWD moisture using an Extech MO220 Wood Moisture Detector. For snakes found within a piece of CWD, all effort was made to place the probe within the crevice exactly where the snake was found and return any displaced portions of the CWD to their original configuration before taking measurements. Furthermore, we kept thermo-hygrometers in place for at least five minutes and allowed microclimate readings to stabilize before we recorded measurements. We measured external ambient temperature and humidity by placing the thermo-hygrometer probes on the ground next to capture locations. We used a tape measure to quantify course woody debris dimensions (i.e. length, height and width) and visually assessed CWD for level of decay. Level of decay is based on a ranked ordinal scale from 1-5, with 1 = freshly fallen and 5 = extensive decay (Table 1).

Table 1. Decay classification scheme for coarse woody debris (CWD). CWD is classified from freshly fallen (1) to almost completely decayed (5). Decay classes adapted from Mohrmann et al. (2010).



	1	**2**	**3**	**4**	**5**
Age	Freshly fallen	Slight decay	Advanced decay	Extensive decay	Extensive decay
Bark	Firmly attached	Loosely attached	Mostly absent	Absent	Absent
Branches	Branches and twigs present	Branches broken, no twigs	Absent	Absent	Absent
Wood texture	Hard, thumbnail cannot penetrate	Hard, thumbnail penetrates	Spongy	Mushy	Disintegrated
Portion on ground	Elevated if branches are present	Part of width touching ground	Entire width of log flat on ground	Sunken partially into ground	Sunken extensively into ground
Percent permeable by *C. amoenus*	0%	0%	25-50%	>50%	100%

We measured biotic variables, such as vegetation and leaf litter cover, within a 1 m^2 quadrat centered over the CWD refuge where the snake was found. We used ground-based digital cover photography to calculate percent canopy cover over refuges and percent cover of ground vegetation surrounding refuges (Reinert 1984, Pekin and Macfarlane 2009). We analyzed digital images with a bespoke Photoshop CS5 algorithm that adjusts the luminance threshold to differentiate between vegetation and background images. This renders a high-contrast image where black pixels represent vegetation, allowing the percentage of vegetation to be accurately calculated from the program's built-in luminance histogram. We also measured soil moisture with an Extech® MO750 soil moisture meter probe inserted to a 10 cm depth at four random points within the quadrat, which were then averaged. Similarly, leaf litter depth was averaged from four random measurements within the quadrat.

For each piece of CWD where a snake was detected ("used sites") we also took the same measurements at a randomly selected piece of CWD ("available sites"). Available sites were defined as the closest piece of CWD found after walking 30 m away from the capture site in a randomly pre-determined direction. We took measurements at both used and available sites within thirty minutes of each other to minimize thermal variation. Biotic attributes can significantly change over time; thus used and available sites were paired—rather than pooled—for data analysis to provide a more accurate comparison of differences between used and available sites across the sampling season.

Statistical Analyses: We used descriptive statistics and one-way analysis of variance (ANOVA) to examine the demographics of captured wormsnakes. We used paired *t*-tests to determine whether microclimate variables within refuges differed between used and available sites, and whether the microclimate within refuges differed from the microclimate immediately outside of the refuges. We also used paired *t*-tests to compare microhabitat variables surrounding CWD at used and available sites. Normality and equality of variance were assessed with normal probability plots, Kolmogorov-Smirnov tests, and Levene's test for equality of variances.

We also compared average daily soil temperatures at a depth of 50 cm to refuge temperatures to determine if they differed throughout the year. Air and soil temperatures were obtained from the USDA Natural Resources Conservation Service's Powder Mill site in Prince George's County, Maryland. A paired *t*-test was used to determine whether temperatures were comparable between Powder Mill and the two field sites. Refuge temperatures were extrapolated for all dates using the equation from a linear regression analysis of measured air and refuge temperatures taken on random dates at the field-sites from March through September. Normality and heteroscedasticity were assessed in residual plots. We then used a Fisher's exact test to compare the number of days soil and extrapolated refuge temperatures were within the snakes' preferred range (16–30°C, *see* Clark 1967, Orr 2006). We completed all statistical analyses in R (version 3.0.2) at $\alpha = 0.05$. Means are reported ± standard error. We used Zar (2009) as a reference for analyses.

Results

Demographics: We found a total of 125 wormsnakes: 88 at Huntley Meadows and 37 at Jug Bay. Of the 118 snakes successfully captured, 58 (49%) were male, 46 (39%) female, and 14 (12%) juvenile. Females had significantly greater mass ($F = 34.1$, $P < 0.001$), longer snout-vent lengths ($F = 65.9$, $P < 0.001$), longer total lengths ($F = 70.1$, $P < 0.001$), and a smaller total length to tail length ratio ($F = 56.6$, $P < 0.001$; Table 2). Eighty percent of measured wormsnakes were probably in their second year, as indicated by snout vent length (between 170 and 230 mm; Willson and Dorcas 2004). Eight percent of captures had visible injuries, such as lacerations or broken ribs, and nine percent of captures were found with a conspecific.

Table 2. Morphometrics of Eastern wormsnakes captured in this study.

Sex/Age	Mean	SE	Min	Max
	Snout Vent Length (cm)			
Female	20.6	0.42	14.7	28.1
Juvenile	11.8	0.42	9.9	14.1
Male	18.8	0.31	13.8	25.2
	Total Length to Tail Length Ratio			
Female	0.14	0	0.11	0.2
Juvenile	0.17	0.01	0.12	0.21
Male	0.18	0	0.15	0.21
	Mass (g)			
Female	7.7	0.44	2	17
Juvenile	2.07	0.2	1	3
Male	6.49	0.27	3	11

Microhabitats: There was no significant difference between used and available sites for refuge temperature ($t = 1.41$, $P > 0.10$) or relative humidity ($t = 0.19$, $P > 0.10$; Table 3). Refuge temperatures were significantly lower than ambient air temperatures ($t = -13.4$, $P < 0.001$), and relative humidity within refuges was significantly higher than outside the refuge ($t = 6.15$, $P < 0.001$).

Table 3. Microclimate characteristics of used and available CWD refugia. There were no significant differences between used and available refugia for any microclimate characteristics.

	Used		**Available**	
	mean	*range*	*mean*	*range*
Temperature (°C)	22.9 ± 2.9	11.8−29.4	22.6 ± 3.0	12.0−28.1
Relative humidity (%)	98.3 ± 4.3	78.2−99.9	98.1 ± 4.5	75.7−99.9
CWD moisture (%)	76.8 ± 28.2	11.5−100	82.2 ± 25.6	13.6−100
CWD temp. (%)	21.3 ± 3.6	7.1−28.0	21.8 ± 3.4	12.1−31.5
Soil moisture (%)	4.1 ± 3.8	0.0−14.3	4.3 ± 3.7	0.0−14.7

There was no significant difference between used and available sites for CWD moisture ($t = -6.21$, $P = 0.090$) or volume ($t = 1.40$, $P = 0.166$). Higher decay classes were used significantly more than expected ($\chi^2 = 66.70$, $P < 0.001$; mean used decay class = 3.92 ± 0.08). There were no significant differences between used and available sites for leaf litter depth ($t = -0.094$, $P = 0.925$), leaf litter cover ($t = -1.22$, $P = 0.224$), vegetation cover ($t = 0.842$, $P = 0.402$) and canopy cover ($t = -1.91$, $P = 0.059$; Table 4).

Table 4. Structural microhabitat characteristics surrounding used and available CWD refugia. There were no significant differences between used and available refugia for any microhabitat characteristics.

	Used		**Available**	
	mean	*range*	*mean*	*range*
Leaf litter depth (cm)	2.2 ± 0.8	0.5−5.3	2.2 ± 0.9	0.0−4.4
Leaf litter cover (%)	99.1 ± 3.8	70.0−100	99.7 ± 2.3	80.0−100
Vegetation cover (%)	14.9 ± 21.9	0.0−93.2	12.7 ± 21.6	0.0−84.3
Canopy cover (%)	75.8 ± 5.9	58.3−95.7	77.1 ± 5.4	53.0−87.4

There was no significant difference in air temperatures between the Powder Mill site where soil measurements were recorded and the field sites ($t = 0.38$, $P = 0.707$). Linear regression showed that refuge temperature increased proportionally as ambient air temperature increased ($R^2 = 0.779$, $F = 160.9$, $P < 0.001$). Refuge temperatures were extrapolated across all dates using Equation 1: Refuge temperature = $(0.6306*A) + 7.0397$, where A equals air temperature at ground level. There were significantly more days at which refuge temperatures fell within the preferred temperature range of wormsnakes compared to soil temperatures ($\chi^2 = 30.36$, $P < 0.001$). Most of the divergence between soil and refuge temperatures occurred during the months of April and May (Figure 3).

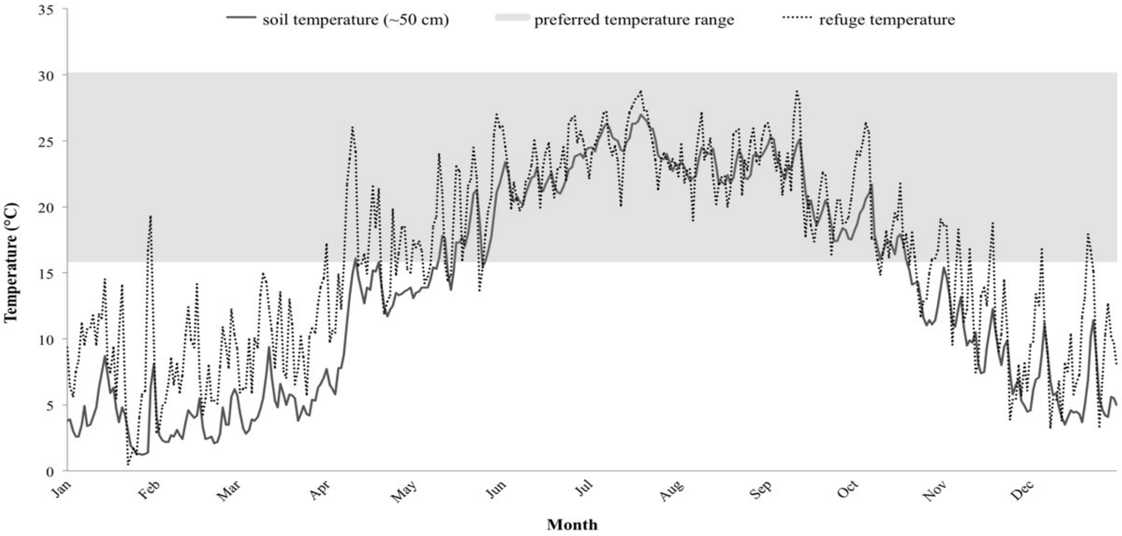


Figure 3. Refuge and soil temperature (~50 cm depth) compared to preferred temperature range for Wormsnakes in this study.

Discussion

CWD, Temperature and Humidity: CWD refuges had uniform microclimates at both used and available sites, making it unlikely that wormsnakes select among available CWD refuges based on temperature or humidity. There was also no indication that microhabitat characteristics outside of the refuge differed between used and available sites. However, our results do suggest that wormsnakes select CWD refuges that provide cooler temperatures and higher humidity than ambient external conditions during spring and early summer (i.e. May, June and July), and provide warmer temperatures than the soil during the early spring, when ground temperatures remain below 10°C.

Many snakes in temperate climates thermoregulate behaviorally, using basking to achieve temperatures high enough for activity, and to facilitate gestation, digestion and recovery from illness (Huey et al. 1989, Reinert 1993). Although we encountered two wormsnakes exhibiting what could be considered basking behavior during the course of this study, this species has not been previously reported to bask in direct sunlight, nor does it appear that they use refuges warmer than surrounding air. Thus, our observations conform to the idea that adaptation to a fossorial lifestyle may have shifted thermal optima in wormsnakes toward lower temperatures, as has been shown in other fossorial reptiles (Kamel and Gatten 1983).

While desiccation tolerance in Eastern wormsnakes has not been studied, its congener *C. vermis* (Western wormsnake) showed low resistance to subcutaenous and cloacal water loss when compared to four other small, fossorial snakes (Elick and Sealander 1972). Nearly all used and available refuges had high levels of relative humidity (mean 98.2 ± 0.29), suggesting that Eastern wormsnakes may seek humid refuges within CWD as part of desiccation avoidance behavior. However, the lack of a humidity gradient between different CWD pieces make it impossible to draw definitive conclusions as to whether Eastern wormsnakes occupy CWD refuges because they provide a humid microclimate, or if the humid microclimate is coincidental to other factors guiding selection.

Wormsnakes spend the majority of their time underground, especially during the winter months, which they spend in underground hibernacula (Barbour 1960, Ernst and Ernst 2003). Soils are slower to warm than air (Parton and Logan 1981); thus in early spring soil temperatures may remain below thermal optima for wormsnakes longer than above ground temperatures. Indeed, we found that refuge temperatures rose to within the species' preferred temperature range earlier in the spring than soil temperatures. The discrepancy between soil and refuge temperatures begins in April, which is also the same month that the majority of wormsnakes move from underground hibernacula into CWD refuges in the mid-Atlantic (Creque 2001, Orr 2006). Wormsnakes within refuges can achieve higher mean body temperatures in refuges within CWD than using other substrates (Orr 2006). Thus, we hypothesize that migrating from the soil into CWD refuges during spring may help wormsnakes remain within thermal optima for a greater proportion of the year. Further study is needed to confirm whether thermoregulation is the sole driver for migration between soil and CWD refuges, or if other factors, such as osmoregulation, also play a role.

Conclusions: The only significant difference between used and available CWD refuges was that used refuges were more likely to be found in highly decayed wood, probably due to the increase permeability at high decay classes, enabling a greater proportion of the wood to be used as refuges. Temperatures were significantly lower within refuges than ambient air temperatures. Coarse woody debris refuges were within the optimal temperature range of Eastern wormsnakes for significantly more days per year than refuges underground, indicating that thermoregulation may be the driving factor influencing CWD refuge selection.

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