MICROHABITAT SELECTION IN THE EASTERN WORM SNAKE (CARPHOPHIS AMOENUS) AND A SURVEY OF THE AMPHIBIANS AND REPTILES OF HUNTLEY MEADOWS PARK, ALEXANDRIA, VIRGINIA

by

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LIST OF ABBREVIATIONS AND SYMBOLS

Meters	m
Celsius	C
Hectares	ha
Coarse Woody Debris	CWD
Huntley Meadows Park	HMP
Jug Bay Wetlands Sanctuary	JB

ABSTRACT

MICROHABITAT SELECTION IN THE EASTERN WORM SNAKE (CARPHOPHIS AMOENUS) AND A SURVEY OF THE AMPHIBIANS AND REPTILES OF HUNTLEY MEADOWS PARK, ALEXANDRIA, VIRGINIA

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George Mason University, 2015

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The eastern worm snake (*Carphophis amoenus*) is a fossorial species that often uses coarse woody debris (CWD) refuges when it is not beneath the ground. The purpose of this study was to determine whether CWD the worm snakes select as refugia differ from available CWD within the microhabitat. This study took place at Huntley Meadows Park, Alexandria, Virginia, and Jug Bay Wetlands Sanctuary, Lothian, Maryland. Snakes were found by searching CWD refuges. Microclimate, ground cover and canopy cover measurements were taken at used and available refuges. There was no significant difference between refuges selected by different sexes or by individuals undergoing ecdysis and the general population. Refuge temperatures were significantly lower than ambient air temperatures, but there was no significant difference between the microclimate characteristics of used and available refuges. Used refuges had significantly more decayed CWD and a higher proportion of CWD cover. They were

also more likely to contain active ant colonies and less likely to contain earthworms. CWD refuges were within the optimal temperature range of worm snakes for significantly more days per year than underground, indicating that thermoregulation may be a driving factor guiding microhabitat selection.

In addition to studying worm snakes, the amphibians and reptiles of Huntley Meadows Park were surveyed to determine their status, which was last assessed in 1989. The comprehensive survey extended across the park and included time-constrained searches of coarse woody debris, anuran calling surveys, vernal pool monitoring, visual encounter surveys and a mark-recapture study of Spotted turtles (*Clemmys guttata*). Spotted turtle populations were higher than expected and one new species, Green treefrogs (*Hyla cinerea*), had moved into the park since the previous survey. However, three species of amphibian and five species of reptile have been extirpated from the park: Northern cricket frog (*Acris crepitans*), Upland chorus frog (*Pseudacris feriarum*), Red-spotted newt (*Notophthalmus viridescens*), Ring-neck snake (*Diadophis punctatus*), Ground skink (*Scincella lateralis*), Mole kingsnake (*Lampropeltis calligaster*), Northern brown snake (*Storeria dekayi*), and Red-bellied snake (*Storeria occipitomaculata*). A further two amphibian species (Fowler's toad, *Anaxyrus fowleri,* and Wood frog, *Lithobates sylvaticus*) appear to be on the cusp of extirpation. Factors that may have contributed to the loss of species include a decrease of meadows and wetlands within the park, increased urbanization surrounding the park, the vulnerability of amphibians and reptiles to road effects, and the closing of habitat corridors between the park and other areas of suitable habitat.

CHAPTER ONE: FACTORS AFFECTING MICROHABITAT SELECTION IN THE EASTERN WORM SNAKE (*CARPHOPHIS AMOENUS*)

Introduction

Not all components of a habitat are of equal importance to the survival of a given species (Huey 1991). Hence individuals undertake a process called habitat selection during which they choose portions of the habitat that will—ideally—best suit their physiological needs (Garshelis 2000). Successful habitat selection results in increased survivorship and abundance of the species in the most suitable areas (Jones 2001). To understand what makes a habitat suitable to a species, we must determine the suite of components that guide habitat selection (Hall et al. 1997, Johnson 1980). This can be accomplished by identifying differences between the portions of the habitat used by a species and the characteristics of the overall habitat available to the species (Manly et al. 2002).

Habitat selection can occur at several nested spatial scales (Hall et al. 1997, Johnson 1980). The smallest scale is the microhabitat and encompasses the area needed for an individual to fulfill its immediate physiological needs, such as nutrient acquisition and thermoregulation (Huey 1991). The size of this scale is thus dependent upon the physiological constraints under which a species operates (Morris 1987). For example, ectothermic taxa such as snakes rely largely on external sources of heat to achieve optimal temperatures for physical exertion and digestion (Stevenson et al. 1985). Small snakes are especially sensitive to temperature changes and subcutaneous water loss, which may render them more vulnerable than larger snakes to variable microclimates (Shoemaker and Nagy 1977, Stevenson 1985). Furthermore, fossorial snakes may require lower body temperatures than other reptiles (Gatten and McClung 1981, Kamel and Gatten 1983). Thus small, fossorial snakes may constitute a guild with a unique set of microhabitat requirements. However, few studies have examined habitat selection in this guild.

This study focused on determining the microhabitat requirements of one representative of the guild, the eastern worm snake (*Carphophis amoenus*). *C. amoenus* is a small, fossorial snake found primarily in deciduous forests in the eastern United States (Ernst and Ernst 2003). When not beneath the soil, *C. amoenus* often takes refuge in the interstitial spaces within coarse woody debris (CWD) on the forest floor (Ernst and Ernst 2003). Coarse woody debris is dead wood, such as logs and branches, with a minimum diameter of 7.5 cm (Harmon et al. 1986). Snakes can use the sheltered microclimate found within CWD refugia to help them thermoregulate while concurrently avoiding desiccation and predation (Daltry et al. 1998, Elick and Sealander 1972, Huey et al. 1989, Winne et al. 2001). Thus, these CWD refugia may play an important role in providing a safe, humid, thermally stable microclimate for *C. amoenus* (Barbour et al. 1969, Conant and Collins 1998). Therefore, identifying the characteristics of refugia selected by *C. amoenus* may give insight into an essential component of this species' habitat.

However, previous investigations into the microhabitat preferences of *C. amoenus* primarily used artificial refugia (e.g. plywood coverboards and concrete slabs) to "capture" snakes and thus did not determine what characteristics *C. amoneus* selects for in its natural habitat (Creque 2001, Orr 2006, Russell and Hanlin 1999). Artificial refugia have significantly greater thermal variation than natural refugia (Houze and Chandler 2002), and because snakes follow thermal gradients to refuge sites (Huey et al. 1989), the use of artificial refugia may alter the behavior of *C. amoenus* during the selection process. Furthermore, no studies have examined if other components of the habitat also play a role in microhabitat selection. For instance, microclimate conditions within refugia are influenced by both intrinsic refuge characteristics (e.g. CWD dimensions) and surrounding structural factors, such as canopy cover, leaf litter cover and understory vegetation composition (Kang et al. 2000, Pringle et al. 2003, Webb et al. 2004).

Thus the purpose of this study was to determine whether CWD that *C. amoenus* select as refugia differ from all available CWD within the microhabitat, and to examine how intrinsic and extrinsic factors affect retreat microclimate. I also attempted to answer three complementary questions: 1) Does microhabitat selection vary based on a snake's sex or body condition? 2) Does the microclimate found within CWD correspond more closely with the preferred temperature range of *C. amoenus* than external, ambient conditions? 3) Does the microclimate found within CWD correspond more closely with the preferred temperature range of *C. amoenus* than conditions underground?

Materials and Methods

Study Sites

This study was conducted at two sites: Huntley Meadows Park (HMP) in Alexandria, VA, and Jug Bay Wetlands Sanctuary (JB) in Lothian, MD. HMP is a 630 ha park containing a variety of habitats, including a central 9 ha wetland, meadows, vernal pools and oak-maple forest. HMP is surrounded by a highly developed landscape and has little connectivity with non-urbanized habitat. Conversely, JB is set in a rural area, bordered by agricultural fields and the Patuxent River. At 650 ha, it is similar in size to HMP, but approximately half of that area is comprised of wetlands, with terrestrial habitats consisting of meadows, deciduous forest and mixed forest.

Snake Searches and Measurements

I used a random stratified sampling procedure designed to sample snakes in a representative portion of available habitats. A TIGER/Line® shapefile of each research site was obtained from the US Census Bureau, loaded into QuantumGIS (QGIS 2.0), and overlaid with a vector grid divided into a 300 m x 300 m squares. If a grid square was found to primarily cover habitats known to be unsuitable for worm snakes (e.g. areas with saturated soils), that square was discarded. I used a random point function in QGIS to choose an equal number of search points for each remaining square. Search points were a minimum of 100 m apart to avoid spatial autocorrelation and maintain observation independence by decreasing the likelihood that the same snake would be observed multiple times.

Searches were concentrated in May, June and July, when the snakes are most likely to be found in refugia (Fitch 1956, Willson and Dorcas 2004). Search points were first located using a hand-held GPS device (Garmin eTrex 20), and the area around that point was systematically sampled using time-constrained searches of one person-hour per site. Searches involved looking beneath and within available CWD by rolling over logs and breaking open decaying CWD with my hands. However, I made an effort to keep disturbance of the habitat to a minimum and therefore did not fully dismantle CWD to find snakes. When a snake was found, it was placed in a small mesh bag while microhabitat measurements were taken. Snakes were assessed for injuries, weighed to the nearest 0.2 g using a Pesola® spring scale, and measured for snout-vent length and tail length using a small ruler. Snakes were sexed visually using the ratio of tail length to total length (approximately 0.14 for females and 0.19 for males), with individuals less than 170 mm in total length counted as juveniles (Willson and Dorcas 2004). After measurements were completed, all snakes were immediately released at the point of capture.

Microhabitat Measurements

For each CWD refuge, I measured microclimate characteristics as close as possible to where the snake was found. If a snake was found beneath a piece of CWD, then temperature and relative humidity were taken using a digital thermo-hygrometer probe placed underneath the log. For snakes found within a piece of CWD, all effort was made to place the probe within the crevice where the snake was found and return any displaced portions of the CWD to their original configuration. Probes were kept in place for a minimum of five minutes to allow the microclimate to stabilize before measurements were recorded. Measurements of ambient external conditions were taken by placing the probes on the ground next to the refuge. CWD objects were measured for temperature and moisture content using a probed wood moisture meter. I also used a tape measure to quantify CWD dimensions (i.e. length, height and width) and visually assessed CWD for level of decay (Table 1-1).

Table 1-1. Decay classification scheme for coarse woody debris (CWD). CWD is classified from freshly fallen (1) to almost completely decayed (5). Decay classes adapted from (Province of British Columbia 2010). A piece of CWD is defined as permeable if *Carphophis amoenus* can move through it easily, based on personal observation.

	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%

Microclimate can be influenced by a variety of surrounding habitat characteristics. Therefore a 1 m^2 quadrat, centered over the location where the snake was found, was assessed for leaf litter cover and depth (Moore et al. 2006, Wund et al. 2007). Both percentage canopy cover over the refugia and percent cover of vegetation surrounding the refugia were measured using ground-based digital cover photography (Pekin and Macfarlane 2009, Reinert 1984). I analyzed digital images using an algorithm created for Photoshop CS5 that automatically adjusts the luminance threshold to differentiate between vegetation and the background. 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 (see Appendix A for techniques).

Since moisture in the upper levels of soil can influence the humidity of the surrounding air (Lee and Pielke 1992), soil moisture was measured using a digital soil moisture meter inserted to a depth of 10 cm. Each piece of CWD was also given a binary classification based on the presence/absence of three invertebrate species that could impact selection in *C. amoenus*: 1) earthworms, which are *C. amoenus*'s primary prey (Barbour 1960, Clark 1970, Willson and Dorcas 2004); formicid ants, whose tunnels provide potential escape routes for small snakes (Pisani 2009); and 3) termites, which increase the permeability of CWD (Harmon et al. 1986).

For each snake refuge discovered, a paired piece of CWD was selected by using the closest piece of CWD found after walking ten paces in a direction pre-determined by a list of random compass degrees. The paired site was subject to the same measurement regime as the refuge site. Thus sites that were “used” were compared to matched-pairs of “available” habitat, an increasingly common technique used in habitat selection studies for snakes (Harvey and Weatherhead 2006, Martino et al. 2012, Row and Blouin-Demers 2006, Waldron et al. 2006, Wisler et al. 2008). Measurements were taken at both used and available sites within thirty minutes of each other to minimize thermal variation.

Statistical Analyses

Measurements obtained at used sites were compared to available sites using paired t-tests for continuous data, including appropriate transformations if data were not normally distributed. This paired approach provided a more accurate comparison of habitat differences than a regression model since biotic variables such as ground cover and canopy cover can change dramatically throughout the field season. Chi-square contingency tables were used to assess differences in discrete data between used and available sites (e.g. CWD decay class and presence/absence of invertebrates). Linear regression was used to determine whether there was a relationship between ambient air temperatures and temperatures within refuges.

C. amoenus spends approximately 60% of its time underground (Clark 1967), and is often found at a depth of 30–60 cm (Clark 1970, Grizzell 1949). Migration between above ground refuges and the soil may result from pressure for the snakes to remain within their optimal temperature range. Therefore, temperatures within refuges were compared to soil temperatures 50 cm below ground. This study lacked the resources to implant data loggers deep in the soil; therefore soil temperatures were obtained from the USDA Natural Resources Conservation Service’s (NRCS) Powder Mill site in Prince

George’s County, MD. A paired t-test was used to compare air temperature at the NRCS Powder Mill site with HMP and JB to ensure that they did not significantly differ . A chi-square test was then used to compare the number of days refuge temperatures and soil temperatures were within the snakes’ preferred temperature range of 14–30°C (see Clark 1967).

All statistical analyses were performed in R (version 3.0.2). Maps were made in QGIS (version 2.0.1). Graphs and tables were made in Excel for Mac 2011 (version 14.4.3). An alpha level of 0.05 was used for all tests. Means are reported ± standard error.

Results

The first sampling season (2012) took place exclusively at HMP. Thirty-eight sites were searched for snakes. However, only 15 sites yielded detections (39.5%), resulting in a total of 30 snakes. During the second season (2013), searches were expanded to include JB. Extensive flooding at HMP reduced the number of sites that could be searched from 38 to 28. Twenty-two of the sites (78.6%) resulted in detections, yielding 58 snakes (Figure 1-1). Twelve of the 13 sites at JB (92.3%) resulted in detections, yielding 37 snakes (Figure 1-2).

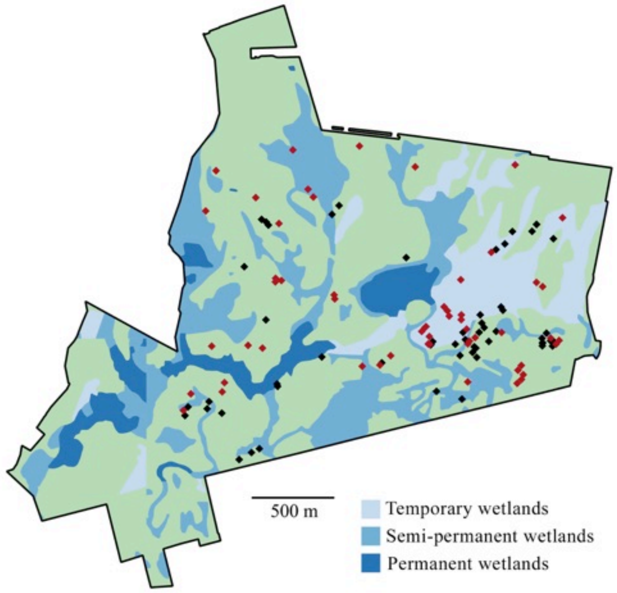

Figure 1-1. Map of Huntley Meadows Park. Snakes found in 2012 represented by red diamonds and in 2013 by black diamonds. Wetland classification based on US Fish and Wildlife Service's National Wetlands Inventory classification scheme.

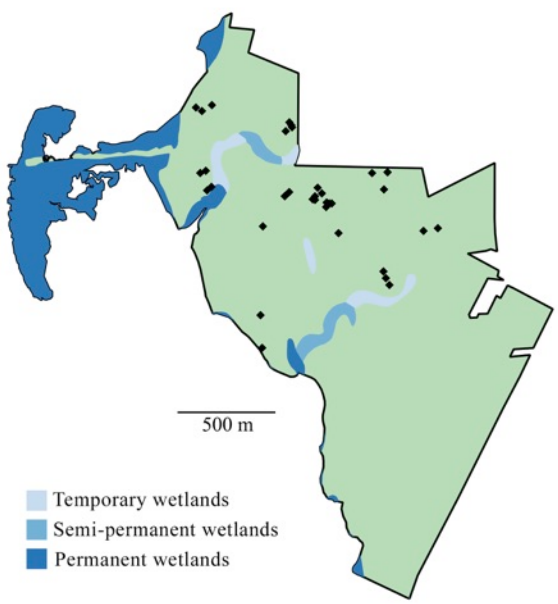

Figure 1-2. Map of Jug Bay Wetlands Sanctuary. Black diamonds represent snakes found in 2013. Southern portion of the park comprises mostly farmland and was not included in searches.

Of the 125 snakes found, 118 were successfully captured. Males comprised 49% of captures, females 39% and juveniles 12%. Females had significantly longer snout-vent lengths (SVL; $t = 3.40$, $df = 95$, $p = 0.001$), greater mass ($t = 2.01$, $df = 91$, $p = 0.0476$), and a smaller total length to tail length ratio ($t = -11.21$, $df = 78$, $p = 2.2e\text{-}16$; Table 1-2). The majority of snakes (80%) were in their second year, as indicated by an SVL of between 170 and 230 mm (Willson and Dorcas 2004).

Table 1-2. Morphological characteristics for captured C. amoneus (*N* = 118). Females had significantly longer snout-vent lengths (SVL; t = 3.40, df = 95, p = 0.001), greater mass (t = 2.01, df = 91, p = 0.0476), and a smaller total length to tail length ratio (t = -11.21, df = 78, p < 2.2e-16) compared to males. Values are reported as means ± standard deviation.

	Proportion of captures	SVL (mm)	Mass (g)	Total/tail length ratio
Females	0.39	206 ± 28	7.5 ± 2.9	0.14 ± 0.02
Males	0.49	188 ± 23	6.5 ± 1.9	0.18 ± 0.01
Juveniles	0.12	118 ± 14	2.0 ± 0.6	0.17 ± 0.03

Refuge temperatures did not significantly differ between the sexes, though males were found across a wider temperature range than females ($t = -0.87$, $df = 96$, $p = 0.144$; Figure 1-3). Twenty percent of snakes captured were undergoing ecdysis, as indicated by clouded eyes and dull scales. There was no significant difference in refuge temperature ($t = -0.77$, $df = 117$, $p = 0.272$) or humidity ($t = -1.42$, $df = 117$, $p = 0.160$) between snakes undergoing ecdysis and those that were not. Eight percent of captures had visible injuries, such as lacerations or broken ribs, and nine percent of captures were found with a conspecific. However, the sample sizes were too low to determine if either injured or gregarious snakes were found at significantly different temperatures than other snakes.

Refuge temperatures were significantly lower than ambient air temperatures (t = -13.38, df = 118, p = 2.2e-16; Figure 1-4). Relative humidity within refugia was significantly higher than outside the refuge (t = 6.15, df = 118, p = 1.116e-08). Regression analysis showed that refuge temperature increased proportionally as ambient air temperature increased (F = 494.67, df = 217, p = 2.2e-16; Figure 1-5). However, there was no significant difference for any microclimate variables between used and available refuges, including refuge temperature (t = 1.41, df = 99, p = 0.160), relative humidity (t = 0.19, df = 98, p = 0.854), CWD temperature (t = 3.79, df = 90, p = 0.628), CWD moisture (t = -6.21, df = 95, p = 0.090) and soil moisture (t = -0.72, df = 96, p = 0.473; Table 1-3).

Table 1-3. Microclimate characteristics of used and available coarse woody debris 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

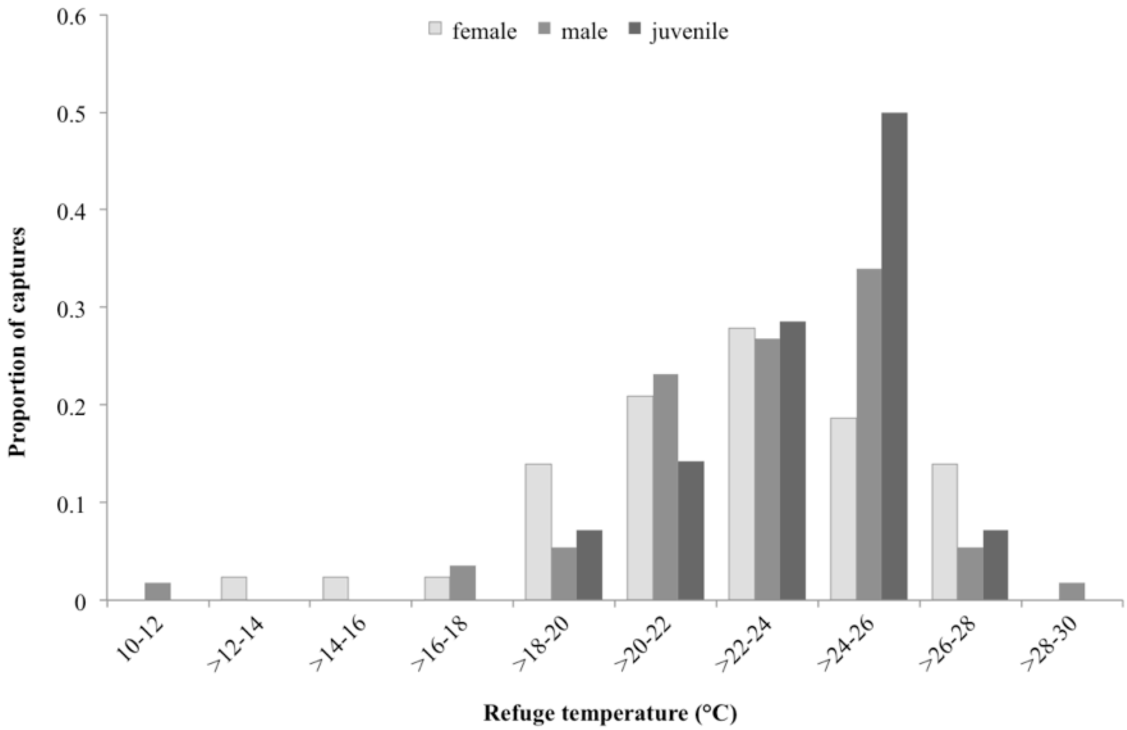

Figure 1-3. Temperatures of refuges where *Carphophis amoenus* were captured, divided by sex. All individuals below 170 mm in total length were considered juveniles. There were no significant differences in refuge temperatures between the sexes.

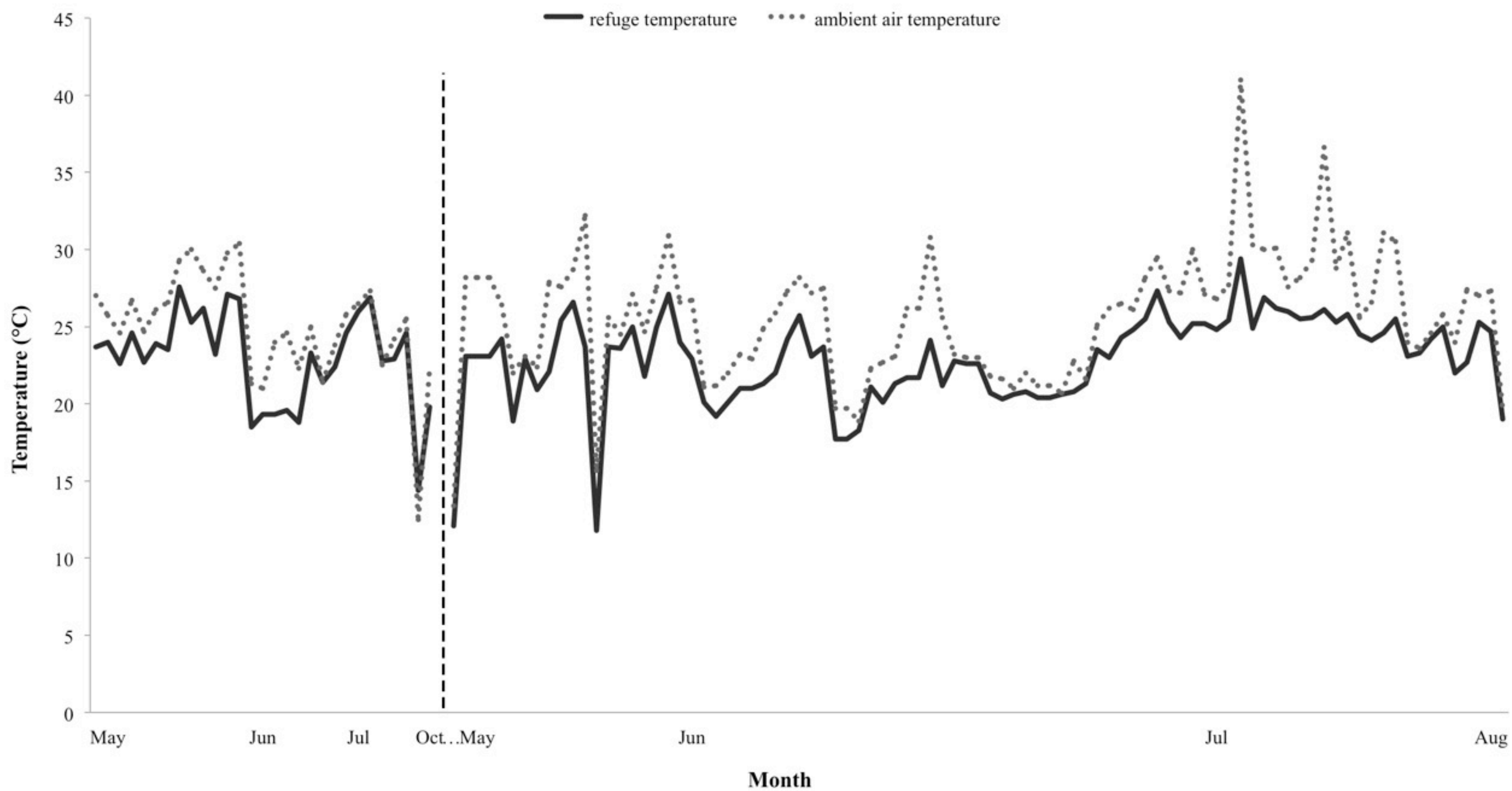

Figure 1-4. Temperatures within refuges and ambient air temperatures throughout both field seasons. Ambient air temperatures were significantly higher than refuge temperatures. Vertical dashed line indicates break between the 2012 (left) and 2013 (right) field seasons.

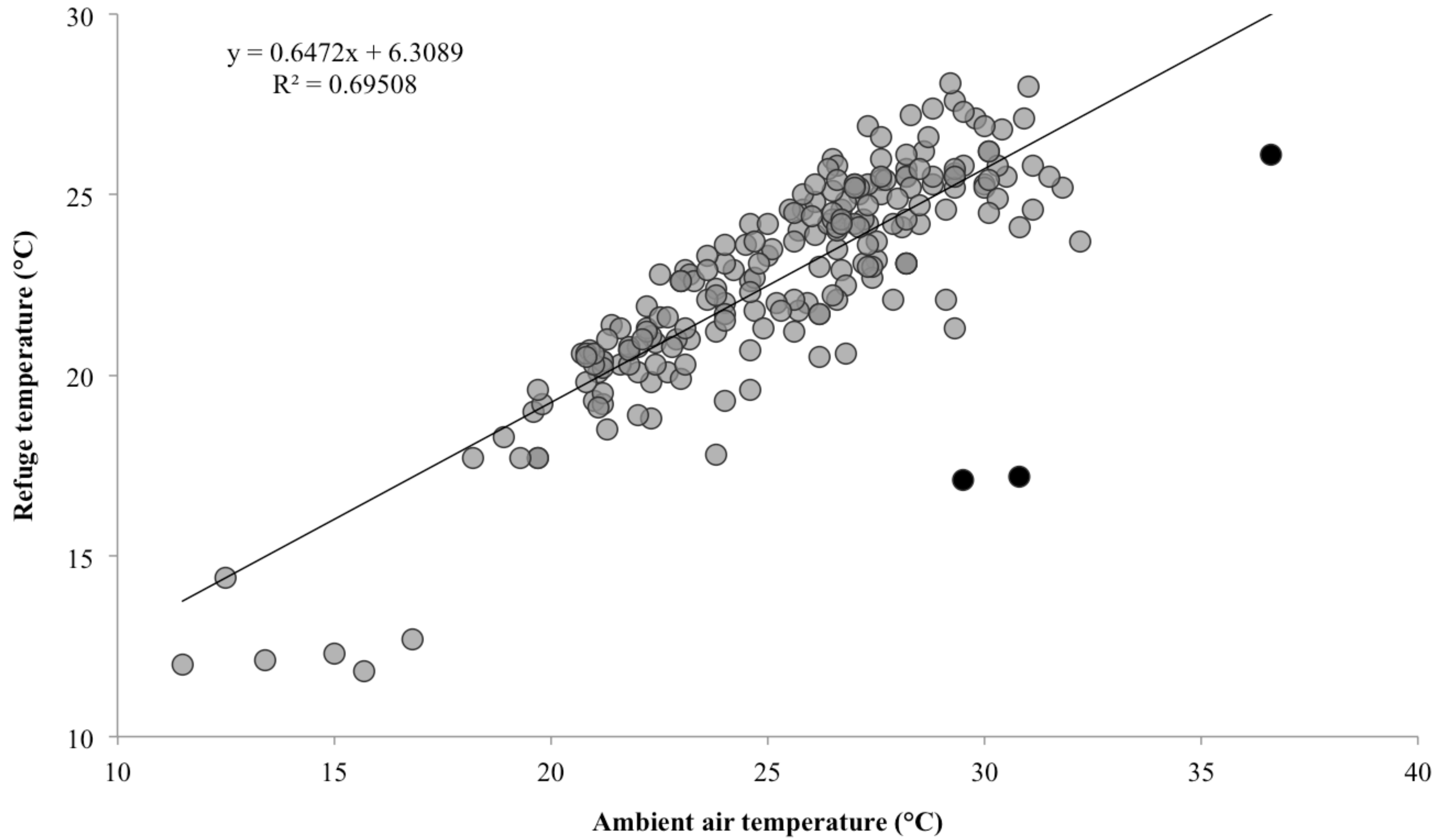

Figure 1-5. Refuge temperatures increased as a function of ambient air temperatures. The three outliers marked in black indicate deviations due to the CWD being exposed to direct sunlight, rather than shaded canopy cover.

There was a significantly higher proportion of the ground covered by coarse woody at refuge sites than paired sites ($t = 3.79$, $df = 97$, $p = 0.0003$). Furthermore, while there was no significant difference in total CWD dimensions between used and available refuges, *C. amoenus* were significantly more likely to be found in pieces of CWD with a larger proportion of the object's surface area touching the ground ($t = 3.95$, $df = 98$, $p = 0.0001$). CWD with higher decay classes were used significantly more than expected ($\chi^2 = 66.70$, $df = 4$, $p = 1.13e-13$; Figure 1-6). There were no significant differences between used and available sites for leaf litter depth and cover, vegetation cover and canopy cover (Table 1-4).

Table 1-4. Structural microhabitat characteristics surrounding used and available coarse woody debris 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

Used sites were significantly more likely to contain ant colonies than available sites ($\chi^2 = 10.18$, $df = 1$, $p = 0.0014$). Conversely, used sites were significantly less likely to contain earthworms than available sites ($\chi^2 = 12.28$, $df = 1$, $p = 0.0005$). There was no significant difference in the presence of termites between used or available sites ($\chi^2 = 3.18$, $df = 1$, $p = 0.075$; Figure 1-7).

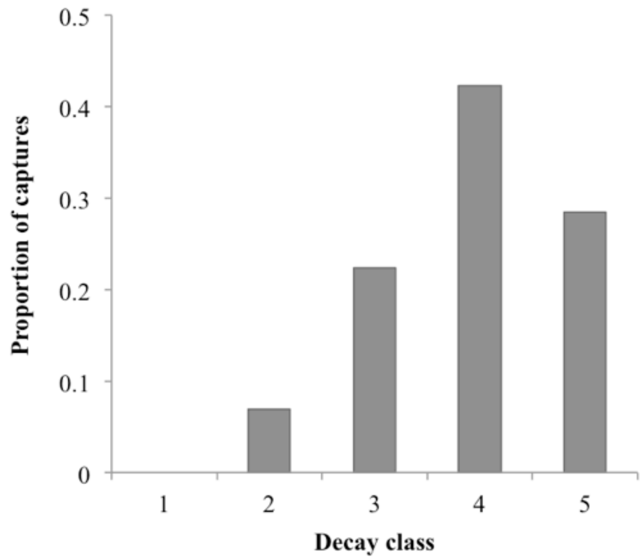

Figure 1-6. Proportion of *C. amoenus* captured in each CWD decay class. *C. amoenus* were significantly more likely to be found occupying CWD in higher decay classes (χ^2 = 66.70, *df* = 4, *p* = 1.13e-13).

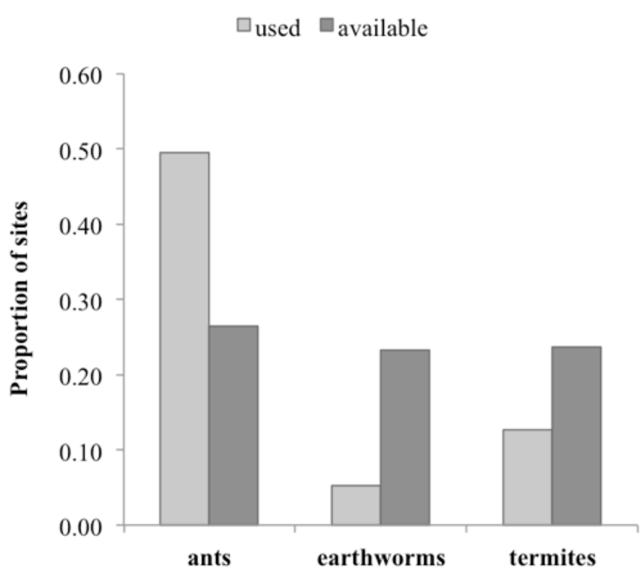

Figure 1-7. Proportion of invertebrates found at used and available refuge sites. *C. amoenus* were significantly more likely to be found with ants (χ^2 = 10.18, *df* = 1, *p* = 0.0014) and less likely to be found with earthworms (χ^2 = 12.28, *df* = 1, *p* = 0.0005). There was no significant difference in termite occurrence at used and available sites.

There was no significant difference between air temperatures at HMP, JB and the NRCS Powder Mill site ($t = 0.21$, $df = 29$, $p = 0.839$), though mean values were slightly higher at NRCS ($\bar{x} = 0.12 \pm 0.59$). There were significantly more days at which refuge temperatures fell within the snakes' preferred temperature range compared to soil temperatures ($\chi^2 = 12.19$, $df = 1$, $p = 0.0005$). Most of the divergence between soil and refuge temperatures occurred during the months of April and May (Figure 1-8).

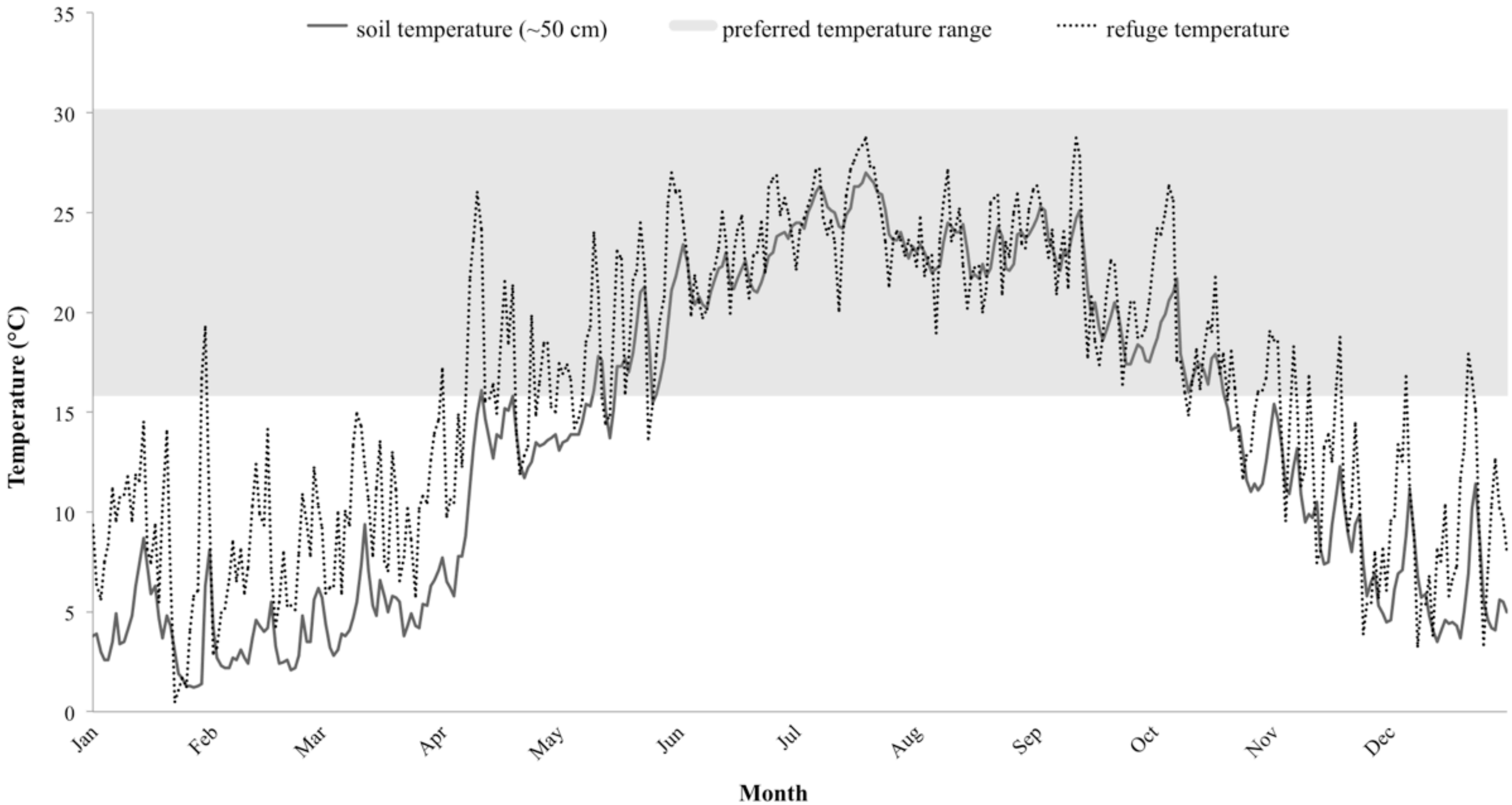

Figure 1-8. Refuge and soil temperature (~50 cm depth) compared to preferred temperature range for *Carphophis amoenus*. There was a significant difference between the number of days refuge temperatures and soil temperatures were within the snakes' preferred temperature range (χ^2 = 12.19, *df* = 1, *p* = 0.0005). Soil temperatures obtained from the USDA Natural Resources Conservation Service's Powder Mill site in Prince George's County, MD. A paired t-test showed no significant differences between air temperature at the USDA site and the study sites (*t* = 0.21, *df* = 29, *p* = 0.839). Refuge temperatures were extrapolated for all dates by calculating refuge temperature from measured air temperatures, based on linear regression analysis of measured air and refuge temperatures (R^2 = 0.65, *df* = 86, *p* < 0.0001). Preferred temperature range of 16–30°C based on previous studies (see Clark 1967, Creque 2001, Orr 2006).

Discussion

In terms of physiological requirements, the traditional paradigm is that snakes in temperate climates bask during periods of cool weather to achieve temperatures high enough for activity, and to facilitate gestation, digestion and recovery from illness (Reinert 1993). Basking is a form of behavioral thermoregulation during which ectotherms absorb heat directly from sunlight or use stored heat radiated from objects, such as rocks (Huey et al. 1989). *C. amoenus* has not been reported to bask in direct sunlight, nor does it appear that they use refuges warmer than the surrounding air. Instead, this study shows that *C. amoenus* occupies refuges that are cooler and more humid than ambient conditions.

There are several factors to consider when assessing these findings. First, habitat selection can only occur if there is variance within the available habitat. In this case, refuges occupied by *C. amoenus* did not significantly differ from the microclimate within random pieces of CWD, indicating that there was a lack of microclimate variability between potential refuges. In particular, humidity varied little between pieces of CWD and remained static across the sampling season, with all pieces having virtually saturated air (98.1 ± 4.53% relative humidity). While desiccation tolerance in *C. amoenus* has not been studied, its congener *C. vermis* had the lowest resistance to subcutaenous and cloacal water loss when compared to four other small, fossorial snakes (Elick and Sealander 1972). Therefore it is possible that *C. amoenus* seeks out humid CWD refugia as a desiccation-avoidance behavior. However, the lack of a humidity gradient between different CWD pieces makes it impossible to draw definitive conclusions as to whether

C. amoenus occupies refugia because they provide a humid microclimate, or if the humid microclimate is coincidental to other factors guiding selection.

It is also possible that *C. amoenus* prefers warmer temperatures, but is prevented from basking due to high predation risk (Lima and Dill 1990, Webb and Whiting 2005, Andersson et al. 2010). *C. amoenus* is a small snake (maximum total length < 290 mm), which relies largely on cryptic coloration and immobility as defense against predation (Ernst and Ernst 2003). When exposed from beneath or within a piece of CWD, 94% of the snakes in this study remained immobile, and escape responses were usually only elicited by tactile stimulation. *C. amoenus* is preyed on primarily by larger snakes (e.g. *Agkistrodon contortrix*, *Lampropeltis getula*, and *Coluber constrictor*; Clark 1970), and songbirds (e.g. *Siala sialis*; Stanback and Mercadante 2000), which are probably unable to access *C. amoenus* within refugia. Hence remaining within refugia ostensibly provides *C. amoenus* protection against predation.

However, during the course of searches, I encountered two *C. amoenus* outside of refugia exhibiting what could be considered basking behavior. Both were oriented with their tails partially beneath CWD and their bodies positioned diagonally outwards from the CWD. Their bodies were held extended, rather than coiled, and they were exposed to the full sun. They did not move when approached, but quickly retreated beneath leaf litter when touched. While this evidence of basking is anecdotal, it is plausible that predation risk (i.e. the "ecology of fear") constrains habitat selection in *C. amoenus* (Brown 1988, Brown et al. 1999). Unfortunately, *C. amoenus*'s small size makes implantation of tracking devices dangerous (Russell and Hanlin 1999), and its cryptic coloration make it difficult to detect outside of refuges. Therefore, it would be difficult to determine if basking is more common in *C. amoenus* than previously assumed, and if the presence of predators in the vicinity or the experience of previous attacks alters *C. amoenus*'s behavior in its natural environment.

Another factor to consider is that refugia may allow *C. amoenus* to fulfill needs unrelated to microclimate. All *C. amoenus* found had recently consumed a meal, as could be seen through their translucent venters by the presence of food darkening the alimentary canal (Barbour 1950). Thus one possibility is that *C. amoenus* uses refugia as hunting grounds for earthworm prey. However, *C. amoenus* were significantly less likely to be found occupying CWD that contained earthworms, with 23% of available sites having earthworms compared to only 5% of used sites (Figure 1-7). Small snakes are only capable of consuming a few earthworms per feeding (Henderson 1970), and CWD refuges with earthworms typically contained over a dozen earthworms, making it unlikely that *C. amoenus* consumed all the earthworms in the refuge. Therefore it is doubtful that the snakes were following chemosensory cues to sites with high prey availability.

In contrast, *C. amoenus* were significantly more likely to be found sharing CWD with active formicid ant colonies. This result was surprising because it was previously reported that *C. amoenus* uses ant nests as hibernacula, but is forced out by ant attacks once the colonies became active (Pisani 2009). I observed no aggressive behavior of ants towards the snakes, even when ant colonies were active. Furthermore, snakes regularly used ant tunnels as escape routes when disturbed. *C. amoenus* may have a commensal relationship with formicid ants whereby rapid escape of *C. amoenus* from predators is facilitated by the presence of ant tunnels. Further study is needed to determine whether this association is mutually beneficial.

It is also vital to consider *C. amoenus*'s fossorial nature when examining habitat selection. *C. amoenus* spend 60% of their time underground, retreating beneath the soil during temperature extremes and droughts (Barbour 1960, Ernst and Ernst 2003). Thus, it may be more relevant to compare microclimate conditions within refugia to those found underground, rather than to ambient air conditions. Refuge temperatures rose to within the species preferred temperature range earlier in the spring than underground temperatures. The discrepancy between soil and refuge temperatures begins in April, which is also the same month that the majority of *C. amoenus* move from hibernacula into refugia in the mid-Atlantic (Creque 2001, Orr 2006). Furthermore, *C. amoenus* within refugia can achieve body temperatures 2.9°C higher than the surrounding refuge (Creque 2001). Thus refugia can help *C. amoenus* significantly increase body temperature and higher temperatures in CWD refugia may allow snakes to become active earlier in the year than if they were confined underground. Additionally, *C. amoenus* achieve higher mean body temperatures from CWD refugia than coverboards or tin sheets (Orr 2006), indicating that occupancy of CWD refuges represent a potentially important sites for thermoregulation.

Conclusion

Coarse woody debris refugia may serve a variety of purposes for *C. amoenus* and none of the factors explored in this study are mutually exclusive (Downes and Shine 1998). The two driving factors guiding microhabitat selection in *C. amoenus* appear to be thermoregulation and reduction of predation risk. The use of refugia may represent a compromise between following a thermal gradient to higher temperatures, which would lead *C. amoenus* above ground, and remaining inaccessible to predators, which would prevent *C. amoenus* from basking in the open. Characteristics of refuges occupied by *C. amoenus* may also indicate selection of “safer” refuges: wider pieces of CWD allow the snake to enter the refuge directly from the soil without exposing itself to predators; higher decay classes allow snakes to burrow deeper within a piece of CWD so that they remain hidden; and tunnels made by ant colonies provide ready escape routes. However, while the results suggest these possibilities, further study is needed to determine the extent to which these factors may impact selection in *C. amoenus*.

CHAPTER TWO: CENSUS OF THE AMPHIBIANS AND REPTILES OF HUNTLEY MEADOWS PARK, ALEXANDRIA, VIRGINIA

Introduction

Amphibians and reptiles are undergoing rapid worldwide declines (Wake and Vredenburg 2008, Böhm et al. 2013), driven largely by habitat loss and fragmentation (Cushman 2006). Urbanization—defined as the increase of human populations above 6.2 individuals/ha (McDonnell et al. 1997)—is a particularly problematic form of habitat loss because it decreases the quality of adjacent habitats, which can increase the sensitivity of populations to stochastic events, constrain dispersal and negatively affect species persistence (Hamer and McDonnell 2008, 2009). Remnant populations residing within the small habitat islands created by urbanization tend to have high mortality rates and depend upon habitat corridors as important avenues of recruitment to prevent extirpation (Davis and Glick 1978, Savard et al. 2000, Bryant 2006, Angold et al. 2006, Delaney et al. 2010). Thus extirpations may increase as corridors close when the surrounding envelope becomes more urbanized (Collinge 1996, McKinney 2008).

Huntley Meadows Park (HMP) provides an ideal case study for the potential effects of urbanization on species persistence within an urban habitat island. HMP is a 630-hectare county park located in the Hybla Valley of Alexandria, Virginia, and is almost completely surrounded by a highly developed residential and commercial landscape. In 1989, a survey of amphibians and reptiles in the park was performed, yielding 16 amphibian species and 25 reptile species (Ernst 1997). However, no follow-up assessment of amphibian and reptile diversity has been performed since that time.

The purpose of this study was to determine whether amphibian and reptile diversity in HMP has changed over time. I also examined whether there were substantial changes to land cover within the park, and within a 1000 m buffer surrounding the park, since the last survey. The ultimate goal of this survey is to provide an account of the status and distribution of species within the park to aid future management decisions.

Materials and Methods

Study Site

The survey took place in Huntley Meadows Park, Alexandria, Virginia (Figure 2-1). The park is currently under the administration of the Fairfax County Park Authority (FCPA). The land was originally used for agriculture until the 1940s, when it was acquired by the U.S. military. Around the same time that the FCPA acquired the land in the 1970s, North American beaver (*Castor canadensis*) moved into the area and dammed Barnyard Run, a stream that runs through the center of the park, creating an eight hectare central wetland. The park consists of a variety of habitats, including meadows, semi-permanent wetlands, vernal pools and deciduous forest. The survey outlined here began in May 2012 and is ongoing.

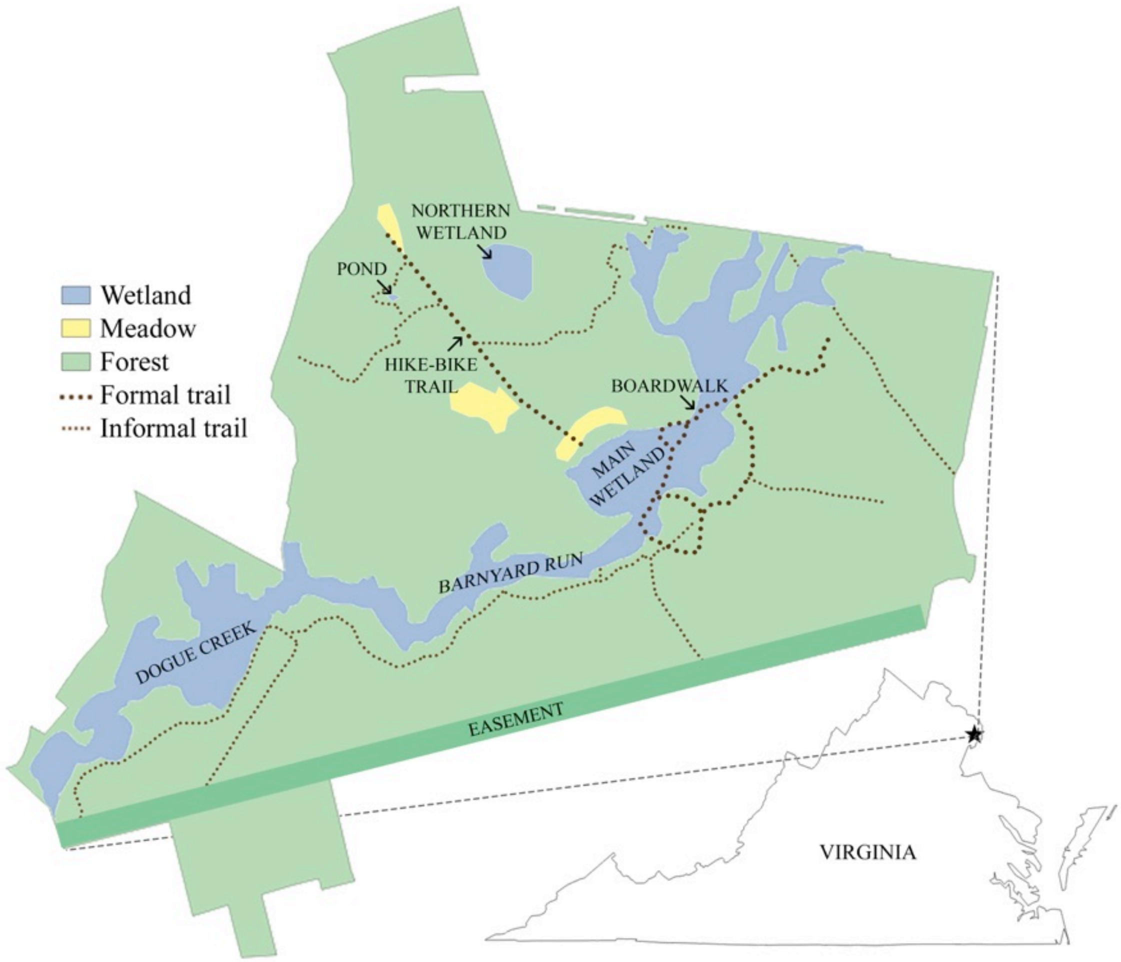

Figure 2-1. Map of Huntley Meadows Park, Alexandria, Virginia, showing the location of major wetlands, meadows, forest, and formal and informal trails surveyed for amphibians and reptiles. The easement is a wet meadow running beneath power lines along the southern edge of the park.

Time-Constrained Searches

Time-constrained searches were conducted from May through September by searching beneath and within coarse woody debris for one-person hour per site during daytime. Search sites were chosen by first stratifying the forested portions of the park into a sampling grid with 300 x 300 m squares. A random point within each square was chosen using the Random Points tool in Quantum GIS (http://www.qgis.org) and was used as the starting point, with searches radiating from that central point. Points found to be unsearchable due to flooding or inaccessibility were replaced with an alternate random point within the same grid, if possible.

Anuran Calling Surveys

An extensive number of calling surveys were conducted at fifteen sites in the park in 2009 and 2010 (Tupper et al. 2012). In 2012, four sites were selected to continue ACS, focusing on the main wetland. ACS were conducted by trained park staff once every two weeks from March through August, beginning once the temperature reached a minimum of 7°C. The ACS regime followed the FrogWatch USA protocol (http://www.aza.org/frogwatch), which consists of a two-minute acclimation period, followed by a three-minute data collection period. Species heard were rated on a three-point call intensity scale to estimate relative abundance: 1) each individual can be heard with no overlap in calling; 2) calls overlap, but individual calls can still be distinguished; and 3) full chorus where individuals cannot be distinguished.

Vernal Pool Monitoring

The southern side of the park has extensive areas covered by vernal pools in the spring, many of which are interconnected. The northern portion of the park also has several vernal pools, including one discrete deep pond that is filled with water year-round during years with above-average rainfall. These fish-free pools represent the only suitable breeding sites for Spotted salamanders (*Ambystoma maculatum*) and Marbled salamanders (*Ambystoma opacum*) in the park, and may be important breeding sites for other amphibians (Petranka and Petranka 1981, Hecnar and M'Closkey 1997). Ten vernal pools were selected for monitoring anuran and salamander breeding activity. All pools chosen had previous observations of salamander breeding activity and were selected to be representative of the different sizes, vegetation regimes and hydroperiods of vernal pools available within the park.

Pools were checked at least once a month for egg masses and larvae, beginning in February and continuing until pools dried or all larvae metamorphosed. Estimated counts of spotted salamander egg masses were conducted by walking slowly along a transect established longitudinally through each pool and counting all visible masses. After egg masses hatched, larvae were collected at one-meter intervals by making a one-meter long sweep with a D-net positioned perpendicular to the transect line. Larvae were then identified to species using dichotomous keys and released at point of capture.

Visual Encounter Surveys

Surveys were conducted by looking for species that could be seen from the boardwalk that runs through the main wetland and from alongside established trails. Unlike time-constrained searches, VES did not involve any habitat disturbance; only species that were readily observable were recorded. Surveys took place at least once a month during the growing season (March-August), but any species observed in these areas during other outside of formal surveys were also recorded. This category includes species observed by park staff members and volunteers when I could confirm the identification from a photograph or video.

Mark-Recapture of Spotted Turtles (*Clemmys guttata*)

A previous study concluded that the *C. guttata* population in the park was under threat of extirpation (Wilson 2002). Therefore, to estimate the current population size I began a mark-recapture study in March 2013. Turtles were found by walking slowly through vernal pools and the northern wetland. Turtles were then hand-captured, sexed visually, given a unique notching pattern and released at point of capture (Plummer and Ferner 2012). A rough estimate of the population size ($\check{N}$) was calculated using the Schnabel index, which uses multiple mark-recapture periods within a sampling period when the population is assumed to be closed (Greenwood 1996):

$$\check{N} = \frac{\sum M_i C_i}{\sum R_i}$$

where M_i is the total number of turtles captured prior to time i, C_i is the total number of turtles captured at time i, and R_i is the number of previously marked animals captured at time i.

Landscape Analyses

Land use change over time was analyzed to identify possible factors affecting habitat within the park and corridors for movement surrounding the park. Land cover surveys for 1992 and 2011 were obtained from the National Land Cover Database (Loveland et al. 1991). To eliminate possibly confounding differences in how land cover categories were calculated during the two years, categories were simplified to represent four types of land cover known to be of significance to amphibians and reptiles: 1) Forest, including deciduous forest, mixed forest, evergreen forest and woody wetlands; 2) Wetland, including open water and wetlands with emergent vegetation; 3) Meadow, including areas covered with mixed grasses and forbs that are grazed or rarely mown; and 4) Developed, including residential neighborhoods, commercial areas, barren ground, and green spaces designed solely for recreational use such as golf courses (Mazerolle and Villard 1999).

Land cover changes were calculated in QGIS using the Landscape ecology statistics plug-in (Jung 2013). Changes within the park were determined by calculating the difference between land cover within the park's boundaries in 1992 and 2011. Cover changes in areas surrounding the park were determined by first drawing a 1000 m buffer around the park's boundaries and subtracting the land cover within the park from the land cover within the buffer. The change in surrounding area was then determined by calculating the difference between land cover between the 1992 and 2011 buffers.

Results

A total of 68 time-controlled searches were conducted, resulting in 229 captures of 13 species (Table 2-1). Eastern red-backed salamanders (*Plethodon cinereus*) were the most common amphibian found, comprising 75.7% (109/144) of captures. Reptiles were represented primarily by Eastern worm snakes (*Carphophis amoenus*), comprising 85.9% (73/85) of captures. Juveniles represented 13.1% (30/229) of all captures, and one Five-lined skink (*Plestiodon fasciatus*) was found guarding a nest of seven eggs.

Eight species were heard during anuran calling surveys in the main wetland (Figure 2-2). Seven species were heard at a call intensity of two or above, indicating abundant populations. Pickerel frogs (*Lithobates palustris*) had the lowest recorded call intensity. Calling surveys failed to detect Fowler's toads (*Anaxyrus fowleri*) and Wood frogs (*Lithobates sylvaticus*).

Table 2-1. Results of time-constrained searches in Huntley Meadows Park.

Amphibians	**Adult**	**Juvenile**
Ambystoma maculatum	3	—
Ambystoma opacum	6	3
Anaxyrus americanus	6	5
Lithobates sphenocephalus	4	—
Plethodon cinereus	99	10
Plethodon cylindraceus	4	—
Pseudacris crucifer		4
Total amphibians	122	22
Reptiles		
Carphophis amoenus	66	7
Pantherophis alleghaniensis	1	—
Plestiodon fasciatus	4	—
Terrapene carolina	1	—
Thamnophis sauritus	5	—
Thamnophis sirtalis		1
Total reptiles	77	8
Total amphibians and reptiles	**199**	**30**

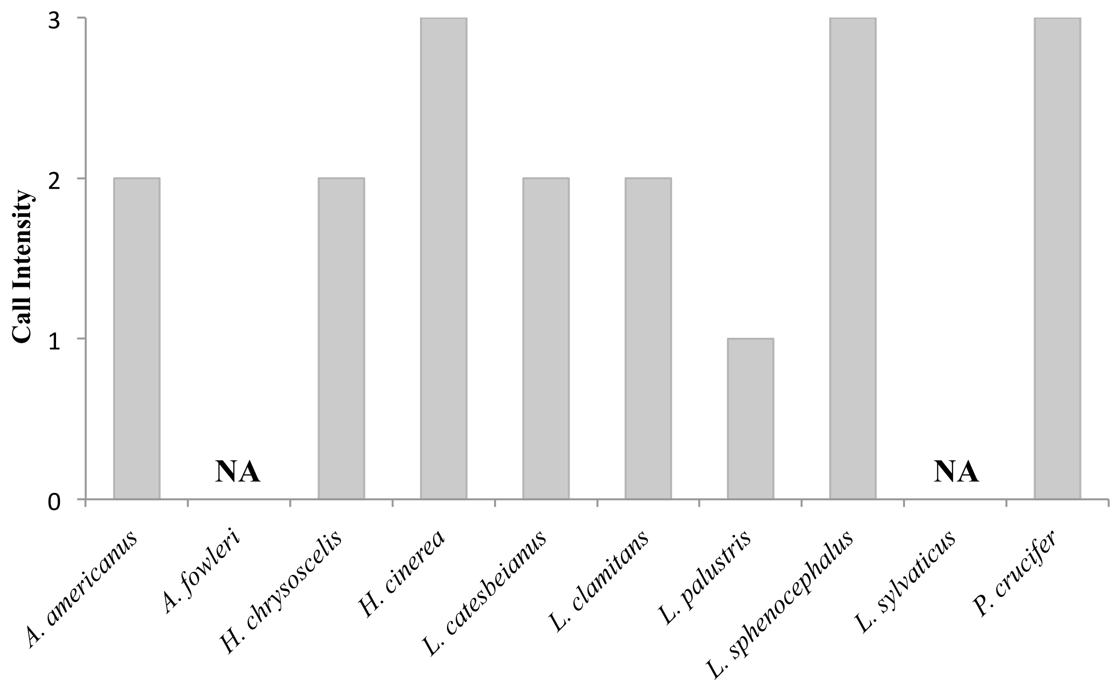

Figure 2-2. Maximum calling intensity heard for ten species during anuran calling surveys in the main wetland. Species were rated on a three-point call intensity scale to estimate relative abundance: 1) each individual can be heard with no overlap in calling; 2) calls overlap, but individual calls can still be distinguished; and 3) full chorus where individuals cannot be distinguished. Species not heard during surveys are marked NA.

Breeding activity was detected in all ten vernal pools. Spotted salamander (*Ambystoma maculatum*) egg masses were found in half of the pools, though only two pools had 10+ egg masses. *Ambystoma maculatum* larvae were found in four pools. Large numbers of egg masses and larvae were found for three anuran species: Southern leopard frogs (*Lithobates sphenocephalus*), Cope's gray treefrogs (*Hyla chrysoscelis*) and American toad (*Anaxyrus americanus*). Spring peepers (*Pseudacris crucifer*) were heard chorusing in vernal pools throughout the park and it is assumed that they are breeding in the pools. During the first year of study, a single egg mass and clutch of *Lithobates* *sylvaticus* larvae were found on the south side of the park. No signs of *L. sylvaticus* were seen the second year of the census.

Visual encounter surveys primarily yielded turtles and larger snakes. Common snapping turtles (*Chelydra serpentina*), Painted turtles (*Chrysemys picta*) and Red-eared sliders (*Trachemys scripta elegans*) were the most commonly observed turtle species. They were usually found basking in the main wetland, though individuals were also encountered in the forest during nesting season. Northern water snakes (*Nerodia sipedon*), Eastern garter snakes (*Thamnophis sirtalis*) and Eastern ribbon snakes (*Thamnophis sauritus*) were the three most commonly observed snake species in the main wetland. All three species were usually found basking on top of emergent vegetation. Rough green snakes (*Opheodrys aestivus*) were also seen regularly in the main wetland hiding within emergent vegetation. Northern racers (*Coluber constrictor*) and Eastern rat snakes (*Pantherophis alleghaniensis*) were only encountered in the forest. Of these species, only *N. sipedon* was never found outside of the wetland.

Four sampling periods were conducted for *Clemmys guttata* during the mating season in March and April. Out of 37 captures, three were in the main wetland, three in the northern wetland and the remainder in a single vernal pool complex. There were no recaptures in the main or northern wetlands, precluding a population analysis (Ryan et al. 2002). Initial analysis of the vernal pool captures yielded a population estimate of 154 turtles. However, due to the sparse number of recaptures (2), the accuracy of that estimate is doubtful (Grimm et al. 2014).

Landscape analyses suggest that there have been substantial changes to land cover between 1992 and 2011 (Figure 2-3). Within the park, forest cover increased slightly (5.7%) while wetland cover decreased (-4.1%). Cover of meadows and developed areas both decreased (-68.8% and -58.23%, respectively). Meadows are the least common habitat, covering only 0.8% of the park (Table 2-2). The area surrounding the park was already highly developed (64.8% coverage) in 1992 and this trend continued, resulting in 77.3% of the land covered by development in 2011 (Table 2-3). All other habitats showed declining coverage, including the almost complete loss of wetlands and meadows, with only 0.33% and 0.03% of the landscape covered by those habitat types, respectively.

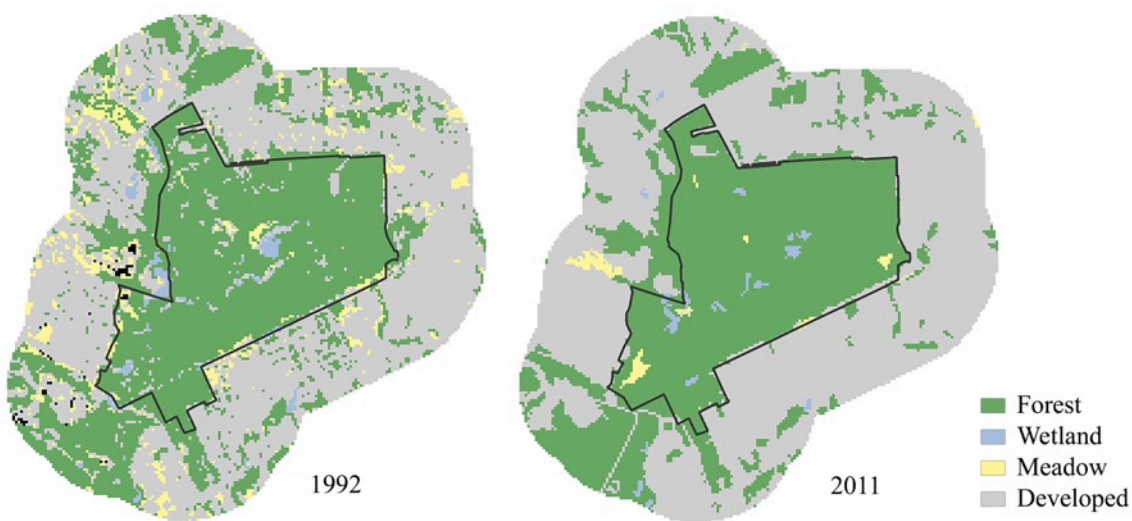

Figure 2-3. Change in and around Huntley Meadows Park between 1992 and 2011. Park boundaries outlined in black. Buffer around park boundaries is 1000 m. Each square (pixel) represents 30 m^2. Within the park, forest increased while wetlands, meadows and developed areas decreased. The buffer surrounding the park experienced increased development and decreased forest, wetlands and meadows.

Table 2-2. Change in land cover from 1992 to 2011 within Huntley Meadows Park.

	Proportion of land cover		
	1992	**2011**	**% Change**
Forest	0.8841	0.9424	5.66%
Wetland	0.0209	0.0202	-4.14%
Meadow	0.0249	0.0079	-68.79%
Developed	0.0700	0.0295	-58.23%

Table 2-3. Change in land cover from 1992 to 2011 in a 1000 m buffer surrounding Huntley Meadows Park.

	Proportion of land cover		
	1992	**2011**	**% Change**
Forest	0.2696	0.2236	-17.23%
Wetland	0.0114	0.0033	-70.56%
Meadow	0.0708	0.0003	-99.59%
Developed	0.6482	0.7727	18.96%

Annotated Species List

Results from this census indicate that there have been changes to the amphibian and reptile communities in Huntley Meadows Park since the last survey in 1989 (Table 2-4). All observations listed below are from this census, unless otherwise indicated. Observations from staff members are from internal park documents or personal communication. Due to previous problems with poaching in the park (D. M. Lawlor, pers. comm.), descriptions of some locations, such as individual vernal pools, are intentionally vague so as not to provide poachers with a map to sensitive species.

Table 2-4. List of species historically recorded in the park and their current status. Adult indicates an adult was observed. Sampling indicates methods species were detected (see Methods for more detail): Time-constrained searches (TCS), anuran calling surveys (ACS), vernal pool monitoring (VPM), visual encounter surveys (VES). Population status estimated through calling intensity during ACS, egg mass counts, or numbers of individuals seen (see annotated species list for more information): Common, species detected regularly; Rare, fewer than 10 detections reported; Extirpated, species undetected for 20+ years; Unknown, more information is needed to accurately assess population status. X: Last observed during this study. Sources for regional trends: a) Weir et al. 2014, b) Brand 2014, c) Virginia Department of Game and Inland Fisheries 2005, d) NatureServe 2014.

	Observed	Sampling	Population status	Regional trend
AMPHIBIANS: Frogs				
Acris crepitans	1990	—	**Extirpated**	Declining[a]
Anaxyrus americanus	X	VES, TCS, ACS	Common	Stable[a]
Anaxyrus fowleri	X	VES, ACS	Rare	Declining[a]
Hyla chrysoscelis	X	VES, ACS, VPM	Common	Increasing[a]
Hyla cinerea	X	VES, TCS, ACS	Common	Stable[a]
Lithobates catesbeianus	X	VES, ACS	Common	Declining[a]
Lithobates clamitans	X	VES, ACS	Common	Declining[a]
Lithobates palustris	X	VES, ACS	Common	Declining[a]
Lithobates sphenocephalus	X	VES, TCS, ACS	Common	Declining[a]
Lithobates sylvaticus	X	ACS, VPM	Rare	Stable[a]
Pseudacris crucifer	X	VES, ACS	Common	Stable[a]
Pseudacris feriarum	1990	—	**Extirpated**	Declining[a]
Salamanders				
Ambystoma maculatum	X	TCS, VPM	Unknown	Stable[b]
Ambystoma opacum	X	TCS, VPM	Unknown	Stable[b]
Notophthalmus viridescens	1989	—	**Extirpated**	Stable[b]
Plethodon cinereus	X	TCS	Common	Unknown
Plethodon cylindraceus	X	VES	Unknown	Unknown

Continued on next page

Table 2-4 continued.

	Observed	Sampling	Population status	Regional trend
REPTILES: Turtles				
Chelydra serpentina	X	VES	Common	Stable[d]
Chrysemys picta	X	VES, TCS	Common	Stable[d]
Clemmys guttata	X	VES	Common	Declining[c]
Glyptemys insculpta	2008	—	Unknown	Declining[c]
Kinosternon subrubrum	X	VES	Unknown	Unknown
Pseudemys rubriventris	X	VES	Rare	Declining[d]
Sternotherus odoratus	X	VES	Unknown	Unknown
Terrapene carolina	X	TCS, VPM	Common	Declining[c]
Trachemys scripta elegans	X	VES	Common	Invasive[c]
Lizards				
Plestiodon fasciatus	X	VES, TCS	Common	Stable[d]
Plestiodon laticeps	Unknown	VES	Unknown	Stable[d]
Scincella lateralis	1989	—	**Extirpated**	Stable[d]
Snakes				
Carphophis amoenus	X	TCS, CB	Common	Stable[d]
Coluber constrictor	X	VES	Common	Stable[d]
Diadophis punctatus	1989	—	**Extirpated**	Unknown
Heterodon platirhinos	X	VES	Common	Declining[c]
Lampropeltis calligaster	1984		**Extirpated**	Unknown
Lampropeltis getula	X	CB	Rare	Unknown
Nerodia sipedon	X	VES	Common	Stable[d]
Opheodrys aestivus	X	VES	Common	Stable[d]
Pantherophis alleghaniensis	X	VES	Common	Unknown
Storeria dekayi	1989	—	**Extirpated**	Stable[d]
Storeria occipitomaculata	1988	—	**Extirpated**	Stable[d]
Thamnophis sauritus	X	VES	Common	Stable[d]
Thamnophis sirtalis	X	VES, TCS, VPM	Common	Stable[d]

Frogs

American toad (*Anaxyrus americanus*). *Anaxyrus americanus* are abundant and have a range encompassing the entire park. Choruses of *A. americanus* were heard during ACS in all locations surveyed. Night surveys led to many incidental encounters on the hike-bike trail north of the wetland and multiple trails south of the wetland. *A. americanus* may preferentially use trails to hunt and are often found sitting motionless next to a line of ants or other prey. They were also found during the day on trails and on the wetland boardwalk. *A. americanus* eggs and larvae were observed in vernal pools on the easement and in the main wetland.

Fowler's toad (*Anaxyrus fowleri*). While *A. fowleri* was considered very common when the park was founded (Division of Conservation 1975), their current status and range has been difficult to ascertain due to their similarity to *A. americanus*. Several individuals observed on the northern side of the park shared characteristics with both *A. fowleri* and *A. americanus* and may represent hybrids between the two species (Blair 1941). ACS did not detect any *A. fowleri*, and while staff members and volunteers have claimed to hear them calling, these detections have not been confirmed with a sighting. *A. fowleri* are unlikely to have a large population in the park. Competition and hybridization with the more populous *A. americanus* may soon lead to the extirpation of *A. fowleri* (Zweifel 1968, Jones 1973).

Eastern cricket frog (*Acris crepitans*). This species was considered common in the park in 1975 and is known to occur at other sites in the county (Mitchell and Reay 1999). However, the last confirmed sighting of this species in the park occurred in 1990 and no ACS performed in the last six years have detected them. *A. crepitans* has been extirpated from the park.

Cope's gray treefrog (*Hyla chrysoscelis*). *Hyla chrysoscelis* are abundant in the park. They were detected in ACS and were heard calling throughout the day in the spring and summer in forested areas north and south of the main wetland. *H. chrysoscelis* in amplexus were observed in puddles along the hike-bike trail on the north side of the park during a night survey, and larvae have been captured in the deep vernal pool referred to as The Pond.

There is some controversy over whether *Hyla versicolor* (gray treefrog) is also present in the park. The two species look identical and have calls that can overlap in pitch during warm weather (Gerhardt and Bee 2006). *H. versicolor* has been confirmed in Fairfax County (Mitchell and Reay 1999), but has not been confirmed during ACS performed by trained individuals in the park.

Green treefrog (*Hyla cinerea*). Unknown in the park before the 1990s, *H. cinerea* are now abundant in the park. They have been found hibernating beneath woody debris during searches south of the main wetland. They were regularly encountered in the main wetland on the leaves of cattails, which provide resting places that match the species' cryptic coloration.

American bullfrog (*Lithobates catesbeianus*). *Lithobates catesbeianus* is abundant in the main wetland. They have been detected frequently in ACS, and adults and larvae were commonly seen in the main wetland. This may be the only area of the park where they breed since their larvae overwinter in water and the main wetland is one of the few places where water is present throughout the year.

Southern leopard frog (*Lithobates sphenocephalus*). *Lithobates sphenocephalus* is very common and probably represent the most populous anuran in the park. Individuals have been seen and heard in every wetland, creek and vernal pool in the park. Egg masses were found in the main wetland, northern wetland and several vernal pools, including those in the easement. Individuals were also found in forested areas far from water, usually sheltering beneath coarse woody debris.

Pickerel frog (*Lithobates palustris*). *Lithobates palustris* is less abundant than *L. sphenocephalus*, but still appear to have a healthy population in the park. They have been observed in the main wetland on numerous occasions and have also been heard during ACS.

Green frog (*Lithobates clamitans*). *Lithobates clamitans* are common in the park. Individuals have been observed in the main wetland and northern wetland, and heard during ACS.

Wood frog (*Lithobates sylvaticus*). In 1975, *L. sylvaticus* was reported to be very common. In recent surveys, *L. sylvaticus* has proven to be elusive and no adults have been observed. However, *L. sylvaticus* was heard calling in the easement during a single ACS (Tupper et al. 2012). *L. sylvaticus* larvae were found in a single vernal pool on the easement. Their population is therefore thought to be small and limited to the southern portion of the park. They are vernal pool specialists that prefer open canopy (Berven and Grudzien 1990), which limits breeding in the park to the easement. However, the easement abuts a suburban neighborhood and has become a dumping ground for trash, such as tires and broken machinery (pers. obs.). It is also tilled annually with a rotary mower, which can cause changes to wetland depth, vegetation composition and water quality (Thomas et al. 2001, Grant 2005). *L. sylvaticus* is considered intolerant of habitat disturbance and thus their decline may be linked to these potential stressors (Julian et al. 2013). It is probable that *L. sylvaticus* will soon be extirpated from the park.

Spring peeper (*Pseudacris crucifer*). *Pseudacris crucifer* are common in all areas of the park. Since *P. crucifer* lays eggs singly on vegetation, no eggs have been seen, but breeding is presumed to be occurring in areas where peepers are heard calling in the spring. Therefore, while metamorphs have only been seen emerging from the main wetland, breeding is thought to occur in the main and northern wetlands as well as the vernal pools on the easement.

Upland chorus frog (*Pseudacris feriarum*). *Pseudacris feriarum* was considered very common in the park when surveyed previously (Division of Conservation 1975) but has now been extirpated. The last recorded observation occurred in 1990. No observations or calls have been heard during any recent ACS. Ernst (1997) reported that *P. feriarum* populations have been declining in Fairfax County since the 1970s, so the extirpation of this species from the park may reflect a broad regional decline.

Salamanders

Spotted salamander (*Ambystoma maculatum*). All observations of adult *A. maculatum* occurred in forested areas adjacent to the south side of the main wetland. However, egg masses were observed in a wider range of the park than adults. While *A.*

maculatum breeding was originally thought to be isolated to the southern side of the park, a few egg masses were found on the northern side, indicating that there is a population north of the main wetland. The breeding season appears to last for at least two months and consists of two waves of activity, beginning first in late February and then again in late April. Egg masses laid during the second wave are less than half the size of the earlier ones, which is consistent with what has been found in other studies (Harris 1980).

It is unclear if *A. maculatum* breeding efforts have resulted in successful recruitment. During the first year of the study, all of the egg masses observed at the largest breeding site were parasitized by chironomid larvae (Insecta: Diptera). Most of the eggs were later found to be non-viable. Furthermore, egg masses were smaller and about half as numerous during the second survey season as compared with the first. No metamorphs were seen during TCS. The population size of *A. maculatum* is difficult to estimate without a mark-recapture study (Bailey et al. 2004), including pitfall trapping around vernal pools to intercept emerging metamorphs (Schmidt and Pellet 2009).

Marbled salamander (*Ambystoma opacum*). Only six adults have been observed, all on the south side of the park. Four breeding sites have been identified so far: two on the south side and two on the north side of the park. Large numbers of larvae were captured at each site, but it is unknown what proportion is able to metamorphose successfully. Like *A. maculatum*, the *A. opacum* population would benefit from further study to help assess their population status.

Red-spotted newt (*Notophthalmus viridescens*). This species is known in the park from a single observation in 1989 (Ernst 1997). While it is possible that a small population may exist, *N. viridescens* has probably been extirpated from the park.

Eastern red-backed salamander (*Plethodon cinereus*). *Plethodon cinereus* is by far the most abundant salamander species in the park. It was the most common amphibian species encountered during TCS. Less than 1% of the population consists of lead-backed color morphs. Four individuals were found with an unusual color variation in which the dorsal stripe is white. Other individuals with this color variation have been found in Fairfax County (John White 2012, pers. comm.) and in Anne Arundel County, MD (pers. obs.), but only a single observation of this color morph has been reported in the literature (Test and Bingham 1948).

White-spotted slimy salamander (*Plethodon cylindraceus*). This species was first observed by a park staff member in 1989. Observations have been haphazard, with no individuals seen for two years and then three found within one meter of each other on a single day. It is uncertain whether the paucity of sightings is due to a small population or low detection probability. *P. cylindraceus* is thought to be declining range-wide (Highton 2005), so their rarity may be symptomatic of a larger pattern of decline.

Turtles

Common snapping turtle (*Chelydra serpentina*). *Chelydra serpentina* is very common in all wet areas of the park. Individuals are particularly abundant in the main wetland, where they have been seen preying on adult *Fulica americana* (American coots), *Aix sponsa* (wood ducks) and *Ardea herodias* (great blue herons). The *C.*

serpentina population is so high that they may be having a detrimental impact on waterfowl populations in the park (D. M. Lawlor 2013, pers. comm.). Outside of the main wetland, snapping turtles were found in vernal pools and streams. Mating activity and juveniles were observed in the main wetland.

Painted turtle (*Chrysemys picta*). *Chrysemys picta* is common in the main wetland, where it is usually found basking on logs in the water. Mating has been witnessed in the main wetland. A juvenile *C. picta* was found on the hike-bike trail north of the wetland and an adult on an informal trail south of the wetland.

Spotted turtle (*Clemmys guttata*). *Clemmys guttata* was once considered under threat of extirpation from the park (Wilson 2002), but it currently seems to have a healthy population. This species is listed as Tier III (High Conservation Need) in Virginia and thus the park's population may represent an important stronghold for the species in the state (Mitchell 1994). Adults have been found in vernal pools throughout the park, as well as the main wetland and northern wetland. Mating was observed in vernal pools, but because they disperse into the forest to breed, their current nesting locations are unknown and no juveniles have been seen. A previous radiotracking study performed in the park focused on a handful of individuals in the northern wetland (Wilson 2002), but it is not known where this species nests on the southern side of the park, where the majority of the population reside. This species may benefit from further study to determine which areas of the park they use for nesting to ensure these areas are protected from disturbance.

Wood turtle (*Glyptemys insculpta*). *Glyptemys insculpta* observations have been few and far between. Park staff members found wood turtles in 1996, 1997 and 2008, amounting to a total of four turtles observed, including one juvenile and one dead adult. HMP represents the edge of this species' known range (Mitchell and Reay 1999). It is unknown whether *G. insculpta* is permanently resident in the park.

Eastern mud turtle (*Kinosternon subrubrum*). A few *K. subrubrum* were observed basking in the main wetland by park staff. These small turtles spend a large portion of the year on land, are cryptically colored and difficult to find (Gibbons 1983), making it difficult to accurately estimate their population status without more extensive study.

Northern red-bellied cooter (*Pseudemys rubriventris*). Park visitors have recently supplied photographic evidence of three probable observations of this species. This species is possibly resident in the park in small numbers.

Eastern musk turtle (*Sternotherus odoratus*). A single *Sternotherus odoratus* observation was reported by a member of park staff during the course of the census. These turtles are nocturnal and spend most of their time in the water (Ernst 1986), so it is possible that they have a larger population that has gone undetected.

Eastern box turtle (*Terrapene carolina*). *Terrapene carolina* individuals are seen regularly in the park. Five adults were seen during surveys. Of these, four were found in the forest south of the wetland and one was found basking in a vernal pool. A box turtle nest was found in the forest south of the wetland, indicating that this species is breeding in the park.

Red-eared slider (*Trachemys scripta elegans*). *Trachemys scripta elegans* is native to the midwestern and south-central United States, but has been introduced around the world through the pet trade (Kraus 2009). This species has been naturalized throughout Virginia, and is particularly abundant in Fairfax County (Mitchell and Reay 1999). Their population in the park probably exceeds that of the native *C. picta*. Their numbers have reached an extent where they may be displacing the native turtle population in the main wetland, particularly the smaller *C. guttata*. More study is needed to determine the impact this invasive species may be having on the native turtles in the park.

Lizards

Common five-lined skink (*Plestiodon fasciatus*). *Plestiodon fasciatus* is common in the park. They were most often seen along the boardwalk in the main wetland and in the forested areas directly adjacent to the wetland. Several individuals were observed hibernating beneath logs within several centimeters of *P. cinereus* and *A. maculatum* individuals. Juveniles were observed on numerous occasions and one nest was found within a piece of coarse woody debris.

Broad-headed skink (*Plestiodon laticeps*). *Plestiodon laticeps* looks extremely similar to *P. fasciatus* and the two species are often confused. All individuals thought to be *P. laticeps* that were captured or photographed to allow close observation of characters were found to be *P. fasciatus*. It is therefore uncertain whether *P. laticeps* is actually resident in the park. Closer observation of the skink population is needed to determine whether this species is present.

Little brown skink (*Scincella lateralis*). The last observation of *S. lateralis* occurred in 1989 (Ernst 1997). This species has probably been extirpated from the park.

Snakes

Eastern worm snake (*Carphophis amoenus*). *Carphophis amoenus* is probably the most abundant snake in the park. Searches have turned up approximately 90 individuals, including a dozen gravid females and two hatchlings. The population seems to be highest on the south side of the park where there are more mature trees and less undergrowth, but individuals have also been found on the north side of the park.

Northern racer (*Coluber constrictor*). *Coluber constrictor* is common in the park. They are regularly encountered during the summer months in the forest south of the wetland. One individual was also found under an old coverboard.

Northern ring-neck snake (*Diadophis punctatus*). A single observation of *Diadophis punctatus* was reported in 1989 (Ernst 1997). This species is known to share similar habitat preferences with *C. amoenus* (Willson and Dorcas 2004), but no *D. punctatus* were found despite extensive searches. If there is a population in the park, it is very small, but mostly likely this species has been extirpated.

Eastern hog-nosed snake (*Heterodon platirhinos*). Observations of *H. platirhinos* were reported by park staff on at least six occasions during the census. One adult was found run over by a construction truck on the hike-bike trail. These snakes were found primarily in the meadows, though one was observed swimming through the main wetland. They have also been observed feeding on American toadlets emerging from the north side of the main wetland.

Eastern kingsnake (*Lampropeltis getula*). This species is rare in the park and was only observed a single time during the course of this census, beneath a coverboard left from a previous study. *L. getula* is likely under threat of extirpation from the park.

Protected populations of *L. getula* are also undergoing declines for unknown reasons (Winne et al. 2007), so factors affecting the species as a whole may be contributing to their decline in the park.

Mole kingsnake (*Lampropeltis calligaster*). The last *L. calligaster* was observed by a park staff member in 1984. This species is fossorial and difficult to detect (Fitch 1993), but considering that it has not been seen for 30 years, it has mostly likely been extirpated from the park.

Northern water snake (*Nerodia sipedon*). *Nerodia sipedon* is common in the park and regularly seen basking in the main wetland. Breeding and juveniles were also observed in the main wetland.

Rough green snake (*Opheodrys aestivus*). *Opheodrys aestivus* are common in the park, though they are difficult to detect due to their excellent cryptic coloration. Individuals were found in the main wetland as well as in forested areas around the park.

Eastern rat snake (*Pantherophis alleghaniensis*). *Pantherophis alleghaniensis* is common and seen regularly in the forest south of the wetland. No juveniles were seen during the course of the census.

Northern brown snake (*Storeria dekayi*). The last observation of *Storeria dekayi* was in 1989. This species has probably been extirpated from the park.

Northern red-bellied snake (*Storeria occipitomaculata*). *Storeria occipitomaculata* has only been observed in the park once, in 1988. This species has probably been extirpated from the park.

Common ribbon snake (*Thamnophis sauritis*). *Thamnophis sauritis* were regularly seen basking in the main wetland. A juvenile was found on one of the informal trails on the south side of the wetland and another was found beneath a piece of coarse woody debris.

Eastern garter snake (*Thamnophis sirtalis*). *Thamnophis sirtalis* is common in the park and regularly found in the main wetland and the adjoining forest. One *T. sirtalis* was also found swimming in a vernal pool. Young *T. sirtalis* were found beneath coarse woody debris in the fall.

Discussion

Three species of amphibian (*Acris crepitans*, *Pseudacris feriarum, Notophthalmus viridescens*) and five species of reptile (*Diadophis punctatus, Scincella lateralis, Lampropeltis calligaster, Storeria dekayi* and *Storeria occipitomaculata*) have not been observed at Huntley Meadows Park for 20 years or more. A further two amphibian species (*Anaxyrus fowleri* and *Lithobates sylvaticus*) appear to be on the cusp of extirpation. It is possible that these species have low detection probabilities, resulting in false absences (MacKenzie 2005). However, cursory surveys of other parks in the region with similar habitats have yielded detections of six of the ten species (*Acris crepitans*, *Anaxyrus fowleri*, *Pseudacris feriarum, Lithobates sylvaticus, Notophthalmus viridescens,* and *Diadophis punctatus*) not detected in HMP during extensive surveys (pers. obs.), making low detection probabilities an unlikely explanation.

Furthermore, since detection probability decreases with decreasing abundance, false absences are more common in species with very small population sizes (Gu and

Swihart 2004). Therefore even if undetected species are still technically extant, they are probably "ecologically extinct" within the park and lack the minimum viable population size to avoid extirpation in the near future (Reed et al. 2003, Ladle and Jepson 2008). Thus failure to detect these species is probably still indicative of serious population declines.

Due to the lack of fine-scale information regarding habitat quality and species abundances over time, it is beyond the scope of this study to pinpoint the causal factors behind the species declines at HMP. However, it is possible to identify possible contributors to the loss of approximately 20% of the park's amphibian and reptile diversity. One of these factors could be a marked change in habitat within the park. The largest change to land cover over time was an increase in forest cover from 88.4% to 94.2%. Landscape changes have differential effects on species depending on their habitat preferences, but in general amphibian richness increases as forest cover increases (Cushman 2006). For example, *Ambystoma maculatum, Lithobates sylvaticus, Pseudacris crucifer* and *Notophthalmus viridiscens* occupancy and abundance are negatively impacted when forest cover around breeding ponds drops below 60% (Skelly et al. 2002, Homan et al. 2004, Herrmann et al. 2005, Eigenbrod et al. 2008). In contrast, many reptile species can benefit from decreased forest cover because it allows greater light penetration for enhanced thermoregulation (Greenberg 2001). However, the small species extirpated at HMP (*Diadophis punctatus, Scincella lateralis, Storeria dekayi* and *Storeria occipitomaculata*) all benefit from greater forest cover (Enge and Marion 1986, Willson and Dorcas 2004, Todd and Andrews 2008). Therefore the increase in forest cover at HMP is unlikely to have contributed to the decline of these species.

The park also suffered losses of wetlands (-4.1%) and meadows (-68.79%). Wetland losses are small enough that they may be an artifact of the relatively low resolution ($30m^2$) of available land cover data, or reflect a temporary reduction in wetland area resulting from an extreme drought event in 2011 (Wang et al. 2014). However, meadow losses are large enough that they probably represent a true change in land cover. Meadows in HMP are a man-made habitat maintained through periodic brush removal and controlled burns. Meadows are preferred habitats for three species that have declined or been extirpated from the park (*Lampropeltis calligaster, Lampropeltis getula, Storeria occipitomaculata*; Table 2-5), but because these species also use forests it is unclear whether meadow losses alone would significantly impact their abundance.

Outside of the park, forest, meadows and wetlands all decreased, while development increased. These changes could have several detrimental effects on amphibians and reptiles. Development, in particular, has significant direct effects, causing increased mortality through road kill (Forman and Alexander 1998, Fahrig and Rytwinski 2009). Amphibians dispersing between wetlands and female turtles migrating to nesting sites are particularly vulnerable to road mortality (Steen et al. 2006, Glista et al. 2008, Langen et al. 2009). Roads can also act as physical barriers, with many snake species and small pond turtles avoiding road crossings altogether (Gibbs and Shriver 2002, Andrews et al. 2005).

Table 2-5. Habitat preferences for amphibians and reptiles found in Huntley Meadows Park. Three circles indicate a preferred habitat, while one circle indicates a habitat that the species will use when preferred habitat is not available (Conant and Collins 1998, Beane et al. 2010).

	Wetlands		Meadow		Forest		
	Permanent	Ephemeral	Wet	Dry	Deciduous	Mixed	Evergreen
AMPHIBIANS: Frogs							
Acris crepitans	•••		•		•	•	
Anaxyrus americanus	•••	•••	•		•••	•	
Anaxyrus fowleri	•••	•••			•	•••	•••
Hyla chrysoscelis	•••	•••			•••	•••	
Hyla cinerea	•••				•••		
Lithobates catesbeianus	•••						
Lithobates clamitans	•••	•					
Lithobates palustris	•••		•		•••		
Lithobates sphenocephalus	•••	•••			•••	•	
Lithobates sylvaticus		•••			•••		
Pseudacris crucifer	•••	•••			•••	•	
Pseudacris feriarum	•••	•••			•••	•	
Salamanders							
Ambystoma maculatum		•••			•••	•	
Ambystoma opacum		•••			•••	•	
Notophthalmus viridescens	•••	•••			•••	•	
Plethodon cinereus					•••	•••	
Plethodon cylindraceus					•••	•	
REPTILES: Turtles							
Chelydra serpentina	•••	•			•••	•	
Chrysemys picta	•••				•••		
Clemmys guttata	•	•••	•		•••		
Glyptemys insculpta	•		•	•	•••		
Kinosternon subrubrum	•	•			•••		
Pseudemys rubriventris	•••						
Sternotherus odoratus	•••						
Terrapene carolina		•	•		•••		
Trachemys scripta elegans	•••						
Lizards							
Plestiodon fasciatus					•••		
Plestiodon laticeps					•	•••	
Scincella lateralis					•	•••	•
Snakes							
Carphophis amoenus					•••	•	
Coluber constrictor				•	•••	•	
Diadophis punctatus					•••	•	
Heterodon platirhinos				•	•	•••	•••
Lampropeltis calligaster				•••	•••	•••	•••
Lampropeltis getula			•••	•••	•••	•	
Nerodia sipedon	•••	•					
Opheodrys aestivus					•••	•	
Pantherophis alleghaniensis			•	•	•••	•	
Storeria dekayi					•••	•••	•
Storeria occipitomaculata			•••	•••	•	•••	•••
Thamnophis sauritus	•••	•••	•		•••		
Thamnophis sirtalis	•••	•••			•••		

Development has indirect negative effects on survival as well. For instance, the "road-effect zone" can extend 1000 m or more into surrounding habitats, changing hydrology, increasing sedimentation in waterways, and providing routes for the egress of invasive species (Forman and Alexander 1998). Urbanization also increases the influx of nutrient and chemical pollution into adjacent wetlands (Faulkner 2004), which in turn reduces amphibian breeding success (Richter and Azous 2010). Furthermore, amphibians and reptiles are found in lower abundances in habitats next to areas with high levels of urbanization and high road density (Lehtinen et al. 1999, Rubbo and Kiesecker 2005, Hamer and McDonnell 2009). HMP is almost completely surrounded by roads, placing most of the park within the road-effect zone, and the surrounding area is highly urbanized, making the park particularly vulnerable to anthropogenic disturbances that negatively affect amphibians and reptiles.

The high degree of urbanization surrounding HMP has essentially turned the park into a terrestrial habitat island. Theoretically, species richness should be a function of the area of the island (MacArthur and Wilson 1967, Diamond et al. 1976), but in reality species-area effects differ largely among taxa and habitat types (Debinski and Holt 2000, Cook et al. 2002). For instance, sensitivity to species-area effects depends partly on the inhospitableness of the surrounding matrix and thus fragments surrounded by highly developed matrices have higher rates of species losses than those with patches of suitable habitat interspersed within the matrix (Prugh et al. 2008). An inhospitable matrix holds a smaller pool of potential immigrants and decreases the likelihood of immigrants successfully reaching the fragment (Wilcox and Murphy 1985). Thus species in isolated fragments are at high risk of extirpation from stochastic events and inbreeding depression because they are unable to be "rescued" by immigration (Brown and Kodric-Brown 1977, Gonzalez et al. 1998, Keller and Waller 2002).

While increased development surrounding the park has probably had a negative impact on species richness, it is unrealistic to expect levels of development to significantly decrease in the near future. However, it is possible to make several broad recommendations to ameliorate the effects of development and improve the outlook of species in the park:

1) Create habitat corridors to surrounding parks. Amphibians and reptiles will readily use riparian buffer zones as migration routes, so restoring buffers around streams running between parks could increase landscape connectivity and immigration rates (Bodie 2001, Crawford and Semlitsch 2007, Maritz and Alexander 2007). If possible, buffers should extend a minimum of 150 m on either side of a stream to provide adequate core habitat (Semlitsch and Bodie 2003).
2) Improve water quality within the park and the surrounding area. The high level of development around the park has led to increased sediment and pollution influx into the park's wetlands (D. M. Lawlor, pers. comm.). Restoring riparian buffers around local streams would improve water quality by filtering pollutants and helping sediment settle before it reaches the park (Correll 2005). Forested riparian buffers would need to extend at least 20 m

on either side of a stream to provide a 70% reduction in nitrate and phosphorus influx, and a 90% reduction in sediment load (Lowrance et al. 1997).

3) Create a comprehensive management and monitoring plan for amphibians and reptiles in the park, with a focus on the ten species that are rare or have unknown statuses (Table 4). To prevent further extirpations, these species need to be studied to determine their abundance, fine-scale distribution within the park and factors that may contribute to their declines.

Conclusion

Eight species have most likely been extirpated from Huntley Meadows Park since the land was first acquired by the Fairfax County Park Authority: *Acris crepitans*, *Pseudacris feriarum, Notopthalmus viridescens, Diadophis punctatus, Scincella lateralis, Lampropeltis calligaster rhombomaculata, Storeria dekayi* and *Storeria occipitomaculata*. The loss of these species may be due to a number of factors, such as decreased landscape connectivity, decreased habitat quality within the park, inbreeding depression, and sensitivity to human disturbance. Previously, the state of amphibians and reptiles in Huntley Meadows Park has largely been ignored and no protocols were established to assess their status. This study highlights the need for the park to establish a comprehensive monitoring program to recognize population declines and take steps to prevent further extirpations before they occur. Steps should also be taken to improve the habitat quality around the park by restoring riparian buffers.

APPENDIX

Background

Canopy cover is the proportion of the forest floor covered by the tree canopy when looking vertically from the ground to canopy, while ground cover is the proportion of the forest floor covered by vegetation or debris when looking vertically from directly above the ground (Jennings et al. 1999). There is no single method for measuring canopy cover and common methods (moosehorn, densitometer, point counts, and line-intercept estimates) produce varying results (Fiala et al. 2006). Furthermore, these methods are sensitive to observer bias, further reducing their accuracy (Ganey and Block 1994). Ground cover methods similarly produce significantly different results between methods and observers (Floyd and Anderson 1987).

With the advent of modern digital photography, both canopy cover and ground cover can be quickly, cheaply and easily calculated with digital images. Observers assess ground cover with the same degree of accuracy whether the cover is observed *in situ* or on a photograph (Murphy and Lodge 2002). Thus by replacing observers with simple computer algorithms to assess cover in images, both observer bias and variability between techniques can be eliminated.

Methods

Taking digital images

Digital images were taken using a Panasonic DMC-FZ40 camera (14 megapixels) and the front camera of an iPhone 4 (0.3 megapixels) with all camera settings on automatic. Images taken with the iPhone 4 were blurrier than those from the Panasonic camera, but both produced the same percentages when rounded to a whole number.

For canopy cover images, the camera was placed flat on the ground with the lens pointing upwards. For ground cover images, a measuring tape was placed on the ground across the area and the camera positioned with the lens facing down at a height that allowed the camera to capture an image covering 1 m square in width. Since the height needed to capture the appropriate size image was always the same, the measuring tape was only necessary for the first few sites, after which it became second-nature to hold the camera at the correct height.

Analyzing canopy cover

Both canopy cover and ground cover were analyzed using Adobe Photoshop versions CS5 & CC, though any digital image manipulation program with similar capabilities could be used. The steps below were turned into an algorithm by "recording" them using the program's action recording function. A folder filled with image can be run through the same action automatically.

The image was first adjusted to have a threshold of 170, which I calculated manually by manipulating images to various thresholds to determine the typical value of the sky. This renders a black and white image with white indicating sky and black indicating anything blocking the sky (i.e. tree branches and leaves; Figure A-1). A circular section of the image was then selected, beginning at the center, to match traditional methods that use circular sections to calculate canopy cover. The percentage canopy cover was then available by looking at the luminosity channel in the image's histogram. Steps are listed below with ">" indicating a submenu selection.

Steps in Photoshop CS5 & CC:

1. Image > Adjustments > Threshold > 170
2. Select circular section from center
3. Window > Histogram
4. In histogram window: Channel > Luminosity
5. Calculate % canopy cover: Hover over histogram to get percentile count

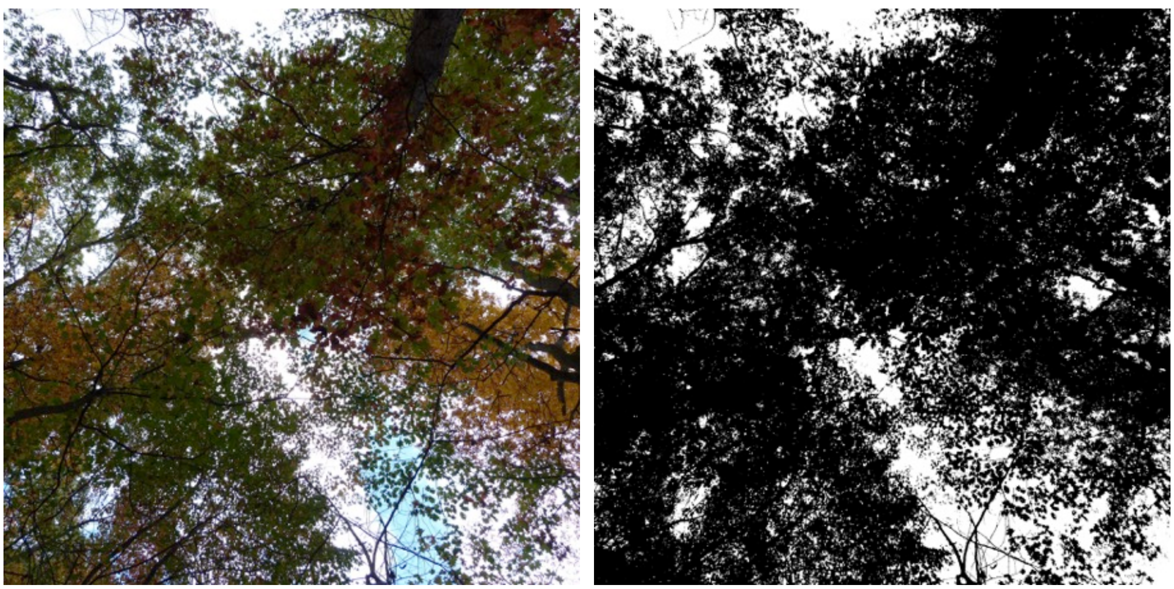

Figure A-1. Digital image of canopy cover before (left) and after (right) manipulation. Black areas represent cover and white areas sky and clouds. Canopy cover calculated to be 77%.

Analyzing ground cover

Ground cover was calculated by squaring the image, selecting green vegetation, and manipulating the threshold to account for variance in the level of greenness of the vegetation. The image was then inverted, rendering green vegetation black and the rest of the image white, allowing the percentage of vegetation to be calculated using the histogram. Accuracy of the algorithm can be assessed visually by overlaying the Alpha layer on top of the original image (Figure A-2). The steps can be altered to suit different colors of vegetation, as long as there is a enough difference between the color of the target item and the background.

Steps in Photoshop CS5:

1. Select square section from center
2. Select > Color range… > Select: Greens
3. Channels > Save selection as channel
4. Make only Alpha 1 visible
5. Click off selection
6. Select Alpha 1 layer
7. Image > Adjustments > Threshold > 20
8. Image > Adjustments > Invert
9. In histogram window: Channel > Alpha 1
10. Calculate total % veg ground cover: hover over histogram to get percentile count

Steps in Photoshop CS5:

1. Select square section from center
2. Select > Color range… > Select: Greens
3. Select > Save selection as… > change name to "Alpha 1" > OK
4. Continue as for CS5, beginning at step 5

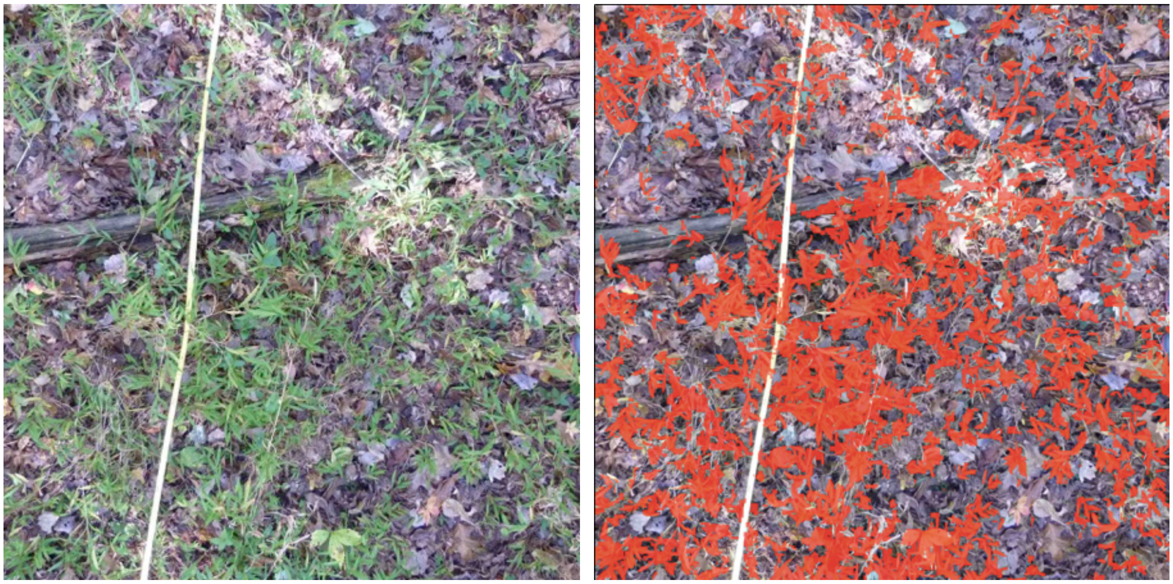

Figure A-2. Digital image of ground cover before (left) and after (right) manipulation. Ground cover consists of a mix of leaf litter, grass, herbaceous vegetation and coarse woody debris. Red areas represent living vegetation. Vegetation calculated at 33% of total ground cover.

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BIOGRAPHY

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