EVALUATING ANURAN RELATIVE ABUNDANCE AND ASSESSING SALAMANDER MOVEMENTS IN PROTECTED AREAS

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ABSTRACT

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Amphibians are important creatures that serve as indicators of wetland health. Recently, there has been a substantial decline in amphibian numbers due to multiple factors including Chytrid fungus and other diseases, habitat destruction and fragmentation, collection, invasive species, and changing climate. While studies on amphibians are on the rise, none have been conducted in the Oak Openings Region of northwest Ohio. This region is a unique mosaic of habitat types ranging from wet prairies, to sand dunes, to oak savannas. These are only three of the fifteen habitat types that encompass the region. In addition, this mixed disturbance landscape is facing urbanization from the north and encroaching agriculture from the south which may put local amphibian communities in jeopardy.

There were three main goals to my study: 1) to determine important landscape, local, and environmental variables to anurans, 2) to determine movement patterns of salamanders, and 3) to determine leaf litter preference for three species of anurans.

First I used frog call surveys along with habitat and environmental measurements to determine what was important to anurans over a two-year period. Different variables were important across spatial scales and these patterns varied temporally. Second, I used fluorescent powder to track salamanders at night. I found that both tiger and spotted salamanders exhibited directionality in movement, but neither this nor any other movement variables measured could be explained by snout-vent length. Third, I used a controlled mescosm experiment to determine leaf litter preferences (maple v. oak) among three Ranid spp. American bullfrogs showed a preference for oak but none of the three species differed significantly from each other.

I would like to dedicate this dissertation to my parents, Chester and Marie Baczynski,

whose love and support I cherish.

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REVIEW INTRODUCTION

Amphibians are unique organisms with most requiring both a wet area for breeding and larval development and a terrestrial area for foraging, summer refugia, and overwintering. While much is known about their use of water the same is not true for our knowledge of their use of land. With little known about these requirements it's not surprising that currently 33% of amphibians are endangered (Stuart et al. 2004). Amphibians are important to our ecosystems. They are used as indicators of wetland health, contribute to a large portion of the biomass of an area, and act as both predators and prey in the food chain (Burton and Likens, 1975; Sparling et al., 2003; Gibbons et al., 2006).

Research took place in various locations in Northwest Ohio including: Oak Openings Preserve Metropark, Secor Metropark, a former ATV site, Maumee State Forest, St. John's/Wintergarden Nature Preserve, and Steidtmann Woods. Northwest Ohio is a unique are of remnant rare ecosystems faced with urbanization encroaching from the north and agriculture from the south (Schetter and Root 2011). The overarching goal of this project was to discern what influences the distribution, diversity and movement of amphibians in the terrestrial landscape. This project incorporated natural field research, a controlled mesocosm experiment, and the use of ArcGIS. Each chapter is formatted for submission to a specific journal.

The goal of Chapter I was to determine important landscape (land use, mean distance to roads, road density, mean distance to 5 nearest wetlands) and local variables (canopy cover, temperature, wind speed, cloud cover, ground cover) to the distribution and diversity of amphibians. This was achieved through multiple methods including frog call surveys, extensive field work, and the use of ArcGIS to create five buffers around each call survey site. Chapter I is formatted for submission to *Landscape Ecology*.

The goal of Chapter II was to determine how far salamanders could be tracked using fluorescent powder, if they distributed uniformly in direction, and if the route they took was linear and if these were related to snout-vent length or mass. This was accomplished through fluorescent powder tracking using a UV light at night. Chapter II is formatted for submission to *Northeastern Naturalist.*

The goal of Chapter III was to determine leaf preference in three Lithobates species: *Lithobates catesbeiana, Lithobates pipiens*, and *Lithobates clamitans melanota.* This was achieved via two methods. The first was a controlled mesocosm experiment comparing preferences of oak or maple leaves for each of the species. The second was a field experiment using frog call surveys and measuring mean leaf depth and mean percent leaf coverage at each call survey site. Chapter III is formatted for submission to *Journal of Herpetology.*

This research contributes to our understanding of the influence of landscape and local factors on the distribution and abundance of amphibians in complex, human-dominated landscapes. With a rapidly changing world, there is a need to better understand how these characteristics affect native species and their ability to thrive in an altered landscape.

CHAPTER I

LOCAL AND LANDSCAPE INFLUENCES ON AMPHIBIAN DIVERSITY AND DISTRIBUTION IN A MIXED-DISTURBANCE LANDSCAPE

Abstract

Nearly one-third of amphibians are threatened or endangered in today's world. With climate change these numbers are likely to increase. It is therefore necessary to investigate what characteristics are important to amphibian success. We wanted to determine what landscape, local, and fragmentation variables affected amphibian relative abundance in a protected area of northwest Ohio. We conducted call surveys, measured various local variables (canopy cover, leaf litter, percent ground coverage), measured fragmentation (distance to roads and wetlands), and measured percent of 15 landcover types within five different buffer sizes around individual breeding pools. Data were analyzed using the Rho test for non-parametric data to find correlations between variables and amphibian relative abundance. We found that species responded differently depending on scale and variables investigated. Some were affected only at small scales (50-100m: Northern green frog), some only at large scales (500-1000m: wood frog), and some across all scales (Northern spring peeper). Local variables were important for multiple species but not the same ones nor in the same direction. Some species responded to many variables (wood frog) while some species responded to few variables (cricket frog). Fragmentation did not have a significant effect on the overall species assemblage. We suggest that sites be managed for all the species in an area, not just one focal species or negative effects on the whole assemblage may occur.

Introduction

Urbanization in the United States has altered wetlands greatly since the arrival of Europeans, drastically reducing the number, availability, and quality of amphibian habitats. Ohio has lost 90% of its wetlands since this time (Dahl 1990) severely reducing the available areas for amphibians to overwinter and breed. Nearly one-third of amphibians are globally threatened and amphibians are the vertebrate group with the highest proportion of species threatened (Stuart et al 2004; Beebee and Griffiths 2005). More than one half of species threatened or endangered in the U.S. are in peril due to urbanization (Czech et al 2000). Urbanization not only fragments the habitat but also increases the likelihood of exotic species establishing, alters hydrology, increases sedimentation, and increases pollution of wetlands (Paul and Meyer 2001; Pickett et al 2001; Miltner et al 2004; McKinney 2006). It is believed that urbanization may be responsible for up to 58% of total wetland losses in the United States (Ehrenfeld 2000). Urbanization has a negative influence on amphibian abundance, especially those that require a greater amount of upland habitat, breed earlier, and are associated with shorter hydroperiods (Rubbo and Kiesecker 2005; Pillsbury and Miller 2008). The focus of this study was to evaluate the influence of local, landscape, and environmental variables on native anuran diversity, abundance, and distribution in the human-dominated landscape of Northwest Ohio.

Several studies have been conducted looking at human impacts on species richness, a measure of healthy populations. Amphibian species richness is higher at ponds surrounded by a lower density of people, at those surrounded by a higher amount of green open space (Pillsbury and Miller 2008; Hamer and Parris 2011), and in areas with greater forest cover (Gibbs 1998; Kolozsvary and Swihart 1999; Houlahan et al 2000). It decreases in areas that are more highly fragmented (Knutson et al 1999; Lehtinen et al 1999). Species were also found to be positively correlated with wetland area and amount of wetlands on adjacent lands (Houlahan and Findlay 2003). Certain species, such as the wood frog (*Rana sylvatica*), avoid open areas such as fields, pastures, and clearcuts (Gibbs 1998; Rothermel and Semlitsch 2002; Regosin et al 2004). The wood frog, eastern American toad (*Bufo americanus*), and northern spring peeper (*Pseudacris crucifer*) prefer deciduous and mixed forests (Waldick et al 1999; Gibbs et al 2005) but see Gagne and Fahrig 2007. The northern leopard frog (*R. pipiens*) was found to be more prevalent in agricultural areas (Gagne and Fahrig 2007).

Roads are also an impediment to amphibian success. There are 13.7 million km of roads in the United States covering approximately 1% of the landscape (Forman 2000). Roads have been shown to decrease amphibian abundance and diversity, restrict movement, reduce gene flow, and increase malformations due to factors such as chemical runoff and pollutants (Gibbs 1998; Vos and Chardon 1998; Turtle 2000; Karraker et al 2008; Marsh et al 2008; Reeves et al 2008). Negative correlations have been found between road density and both amphibian species richness at breeding sites (Findlay et al 2001; Houlahan and Findlay 2003) and anuran pond occupancy (Vos and Chardon 1998).

Agricultural lands have increased dramatically making up approximately 41% of the total available land area in the United States (Anderson and Magleby 1997). Modifications to convert land to agriculture include draining wetlands and clearing upland habitat (Knutson et al 1999; Joly et al 2001). These modifications have had a negative effect on amphibians in the Midwest (Lannoo 1998; Kolozsvary and Swihart

1999), as have the use of pesticides and herbicides. Amphibians have had a challenging time in Ohio crossing the impermeable matrix created by changes in agricultural practices, including the use of feedlots instead of pastures, elimination of fence rows, and using a two year rotation between corn and soybean (Lafferty 1979). Agricultural land has been shown to have a negative impact on amphibian occurrence and species richness as well as increasing population isolation (Piha et al 2007; Greenwald et al 2009). These landscapes do provide open areas for some species that prefer low canopy cover (toads) and woodlots in agricultural areas have proven to be used by some amphibians (Weyrauch and Grubb 2004; Gagne and Fahrig 2007).

Northwest Ohio is a unique area of remnant rare ecosystems (e.g., wet prairies and oak savannas) faced with urbanization encroaching from the north and agriculture from the south (Schetter and Root 2011). Climate change is going to add new dimensions to the loss of suitable amphibian habitat crisis: freshwater ecosystems are one of the systems most at risk (Semlitsch 2000; Parmesan 2006; IPCC 2007). Some species have begun breeding earlier due to warming climates (Beebee 1995; Blaustein et al 2001; Chadwick et al 2006). Amphibians that do not disperse great distances or are habitat generalists fare better in suburban and urban environments compared to their counterparts (Hamer and McDonnell 2008). This means species that specialize on vernal pools are at high risk. Amphibians are declining in number worldwide and it is therefore necessary to identify areas they inhabit to assess what they are using and what needs protection (IUCN 2008). As the climate changes, species' ranges will shift and survival and reproduction will likely be negatively affected by changes in temperature and precipitation patterns along with increased UV-B levels (Semlitsch 2000). Areas amphibians move to as a result of climate change may be lacking in one or more resources/requirements.

Species diversity and relative abundance (RA) are useful variables to measure when trying to assess the importance of different habitats (Gibbs 1998; Kolozsvary and Swihart 1999; Houlahan et al 2000; Gagne and Fahrig 2007). Elucidating these variables allows us to determine which species uses the pond when; are species co-occupying areas where they are usually not found together historically; is a population thriving or barely hanging on; does calling time or duration change if a species is the only one calling as compared to if multiple species are calling, etc. Studying the same sites over multiple years allows one to detect spatial and temporal trends. Frog call surveys are a common method used to assess species diversity and RA in wetland areas. These types of surveys have determined that anuran presence is negatively associated with urban areas (Knutson et al 1999) and that anuran distributions are affected by habitat variables at multiple spatial scales (Price et al 2005). These studies, however, usually do not look at a suite of variable types over time nor do they look at mixed disturbance areas like the Oak Openings Region where fragmentation is high and there are multiple landcover types including oak savannas and wet prairies (Abella et al 2007). The predominant surveying method used involves visiting a site, picking a location where the entire site can be heard, and monitoring it for a set amount of time (usually a three or five minute period). During this time, calling species are identified and RA estimated on a 0 to 3 scale (0=no calls, 3=chorus).

The goal of this study was to evaluate what environmental (temperature, wind speed, cloud cover), local (canopy cover, ground cover, hydroperiod, leaf litter) and landscape (land use, distance to roads and wetlands, length of roads) variables best predict anuran presence and RA in vernal and permanent water bodies of Northwest Ohio. There are no published studies investigating anuran richness and important predictive values in a mixed disturbance landscape that is actively managed for terrestrial species and recreational activities. This provides a unique opportunity to determine important factors for species relative abundance that can later be applied to similar landscapes. There are ten species that occur in this region of Ohio: the eastern American toad, Fowler's toad (*B. fowleri*), grey treefrog (*Hyla versicolor*), northern spring peeper, western chorus frog (*P. triseriata*), American bullfrog (*R. catesbeiana*), northern green frog (*R. clamitans melanota*), northern leopard frog, wood frog and Blanchard's cricket frog (*Acris crepitans blanchardi*). We had four main expectations based on previous findings in the literature. First, we expected that species that prefer forested areas would prefer areas with a higher canopy cover and predicted their RA would be higher in forested areas compared to open areas. Second, we expected that vernal pool species would prefer areas with a shorter hydroperiod and predicted their RA would be negatively correlated with hydroperiod duration. Third, we expected that roads would have a negative effect on anurans and predicted that their RA would be lower in areas with a higher density of roads. Fourth, we expected that wetlands would provide additional habitat for amphibians and predicted that RA would be higher the closer wetlands were to surrounding water bodies where call surveys were done. We expected that the combination of local, landscape, and environmental variables would interact and influence species differently over time.

Methods

Study Sites

All surveys were performed at sites in Wood and Lucas Counties of northwestern Ohio. Two sites were in Wood County (Fig. 1): Steidtmann Woods (32.4 ha) owned by Bowling Green State University and Wintergarden/St. John's Nature Preserve (41.7 ha) owned by Bowling Green Parks and Recreation. Steidtmann Woods is primarily swamp forest with two permanent water bodies and seven vernal pools. The predominant tree species are oak (*Quercus*) and maple (*Acer*) (Ruffer 1961). Wintergarden Nature Preserve consists of forests, prairie meadows, and a wetland area.

The remaining survey sites were in the Oak Openings Region in Lucas County, which is comprised of remnant natural ecosystems set in an urban/agricultural matrix. The Oak Openings region (Fig. 2), (historically 467,000 ha) is highly fragmented with 15 landcover types (Appendix 1) (Schetter and Root 2011). Two sites were at Secor Metropark (Fig. 3) (237.1 ha). It has tall timber, second growth forest, sandy areas, wet lowlands, meadows, and prairies. Two sites were at Maumee State Forest (Fig. 3) (1255.7 ha). In Maumee State forest there are 15 fragmented areas with 799 ha classified as native hardwood, 288 ha are conifer/pine plantations, 146 ha are planted hardwood and 20 ha are wet prairie/wet sedge meadow areas. Seven sites (eight in 2011) were at Oak Openings Preserve Metropark (1523.6 ha). This diverse protected area is composed of the imperiled oak savanna, oak woodland, pin oak flatwoods, sand barrens, and prairies. One site was at a former ATV site (Fig. 4), a 24.6 ha wet prairie surrounded by forest purchased by Toledo Metroparks. All wetlands surveyed were on protected lands with varying levels of management; this ranged from minor trail clearing at Steidtmann

Woods to controlled burns and mowing at Oak Opening Preserve Metropark. All but two (ATV site, Steidtmann Woods) were also used for varying levels of recreation (hunting, fishing, walking trails, childrens' parks).

Frog call surveys

In the spring/summer of 2011 and 2012 we conducted frog call surveys at 15 and 14 sites, respectively, to assess anuran presence, RA (0-3 scale), and diversity in various wetland types of Northwest Ohio. We chose both permanent water bodies and vernal pools where anurans had been heard calling previously (pers. comm. Karen Menard and personal observation). Two of the sites were in Wood County, Ohio; the remaining 12 were in the Oak Openings Region of Lucas County, Ohio. Seven sites were small or open enough that one listening point was sufficient to hear all anurans and eight sites had multiple listening points (>0.2ha and closed canopy). We did not conduct surveys if it was raining or wind was high because anurans are unlikely to call during these conditions (Davis and Menze 2002). We visited each site a minimum of 15 minutes following sunset and waited up to five minutes to hear a call. If no calls were heard by the time five minutes elapsed the site was deemed to have no calling frogs. If a call was heard, we identified calling species and estimated the number heard. Three minutes were spent monitoring sounds after the first call was heard. In 2011 we surveyed each site every two to three weeks from March 21st to July 20th for a total of seven visits per site. In 2012 we surveyed each site every other week from March 13th to July 13th for a total of nine visits per site. All sites were monitored either the same day or within three days with similar weather conditions.

We classified the relative abundance of anurans on the following 0-3 scale (Davis and Menze 2002; Pillsbury and Miller 2008).

0 = no calls heard

1 = individual calls not overlapping

2 = some overlapping calls, but number of individuals calling can be reliably estimated

3 = continuous chorus of calls; individual calls can't be discerned

Local and environmental variables

We measured a number of local habitat-related variables at each of the frog call survey sites. During the summer of 2011 we measured canopy cover at each site via photographs (using a level on a camera) every 40 meters around the perimeter of the water body and within 2 m of the water. We transferred the pictures to a computer, used the ImageJ program (Rasband 2012) to convert the pixels to black and white, and analyzed the percent black as canopy. We averaged the percentages of each canopy measurement to obtain a mean percent canopy cover for each field site. We measured plant diversity at the ground cover level. Using a 1 m x 1 m quadrat every 20 m around the perimeter of the pond (approximately where the land and water met) we estimated the number of species in the quadrat, categorized the species as a graminoid, forb, or shrub/tree, estimated the percent coverage of each species, and calculated a Shannon Diversity index. Every 40 m around the perimeter of the water body we used a quadrat and measured percent leaf litter cover within the quadrat and mean leaf litter depth at five points within the quadrat and obtained a mean value for each site. Length of hydroperiod (days) was measured by visiting sites every two weeks and identifying those that had dried up. If a site was wet one week and dried up two weeks later, we assumed it reached dryness halfway between the two visits. The weather information was obtained from the NOAA website (Toledo Airport weather station) for each survey time and day, including temperature, wind speed, and sky cover. If a survey took place between two weather station readings, the values on either side of the survey were averaged. Data were analyzed in JMP v. 9.0 with a Spearman's rho correlation for nonparametric data to assess the relationship between species' distribution and abundance. Cloud cover was separated into four categories: clear, scattered, broken, and overcast. Mean percent canopy cover, mean percent ground cover, Shannon diversity index, mean leaf litter depth and percent, hydroperiod, temperature, and wind speed were compared with total number of species heard at a site, maximum number of species heard at a site, maximum number of species heard during one night, and RA of each species using Spearman's rho statistic for non-parametric data. Cloud cover was analyzed using a Chi-square test.

Landscape variables

We used ArcGIS (ESRI v 10) to measure multiple landscape level factors at five different buffer distances around survey sites. The nested buffers were spaced 50 m, 100 m, 250 m, 500 m, and 1000 m from the center of the field site; this encompasses a range that extends to approximately four times the mean distance an anuran migrates (review Semlitsch and Bodie 2003). We used a landcover map of the region (Schetter and Root 2011) to determine the percentage of landcover type (of 15 different types) within each buffer. As a proxy for connectivity/fragmentation we also measured distance to five nearest roads, five nearest major roads, and five nearest wetlands (as indicated on the

National Wetland Inventory map) along with the total length of roads within each of the five buffers. Mean distance to five nearest roads, mean distance to five nearest major roads, mean distance to five nearest wetlands, total length of roads within each of the five buffers, and percent landcover type within each of the five buffers, were compared to total number of species heard at a site, maximum number of species heard at a site, maximum number of species heard during one night, and RA of each species using a Spearman's rho test for nonparametric data. A Bonferroni correction was used to account for lack of independence.

Results

Scale and Heterogeneity of Landscape

Percent landcover type varied depending on site with areas varying in levels of heterogeneity (Appendices 2a-2e). At the 50m scale the number of landcover types found at individual sites ranged from 3 to 9. At the 100m scale the number of landcover types found at individual sites ranged 5 to 11. At the 1000m scale six sites had all 15 landcover types within the buffer. We found that for four species landcover type was not important in predicting RA. These were the northern leopard frog, the American bullfrog, the grey treefrog, and the cricket frog. The American toad was only affected at the largest scale, 1000m, with RA positively correlated with turf (Table 1). Wood frog RA was positively affected at larger scales with asphalt significant at 500m and pond significant at 1000m (Table 1). Chorus frog RA was significantly and positively correlated with residential landcover at the 100m scale (Table 1). The spring peeper was the most affected of the species (positively): at 50m with floodplain forest, at 250m with residential and shrub/scrub, and at 500m with swamp (Table 1). The northern green frog was affected positively at smaller scales: at 50m with turf and at 100m with floodplain forest (Table 1). Floodplain forest was the most important of the landcover types, being significantly correlated with species' RA three times. It was followed closely by residential areas which were correlated with species' RA two times. All five scales were equally important: each was found to be significant two times.

Relationships varied temporally with certain landcover types being important in one year but not in the other. For example, the American toad, spring peeper, and Northern green frog were only correlated with landcover types in 2011 while the chorus frog and wood frog were only correlated with landcover types in 2012. This is likely due to the varying environmental variables resulting from one year having an extremely wet spring (2011) and the other year being relatively dry (2012) (Appendix 6).

Local and environmental variables

Environmental variables played an important role for several species (Tables 2-6). Three species RA were negatively affected by temperature: the wood frog, western chorus frog, and spring peeper while three species RA were positively affected by temperature: the grey treefrog, American bullfrog, and northern green frog. Wood frog relative abundance was positively correlated with leaf litter depth (ρ =0.7172, P=0.0039) and percent leaf litter coverage (ρ=0.6178, P= 0.0186). American toad relative abundance was nearly negatively correlated with percent canopy cover (ρ =-0.5167, P=0.0585). Spring peeper relative abundance was negatively correlated with percent ground cover (ρ =-0.5465, P=0.0432). Grey tree frog relative abundance was negatively correlated with percent canopy cover (ρ =-0.5423, *P*=0.0451) and with percent ground cover (ρ =-0.4989, *P* =0.0694) though not significantly. Percent canopy cover was negatively correlated with percent ground cover (ρ =-0.6264, *P*=0.0165). The wood frog (ρ =0.5654, *P*=0.0351), spring peeper (ρ =0.6314, *P*=0.0154), and northern green frog (ρ =0.6616, *P*=0.0100) were all positively correlated with hydroperiod (Appendix 3): the longer the hydroperiod the higher the RA. Hydroperiod was also positively correlated with the mean number of species heard at a site (ρ =0.7444, *P*=0.0023) and total number of species heard at a site (ρ =0.7708, *P*=0.0013). Shannon Diversity index was not correlated with any frog RA. Wind had a negative effect on the American toad in 2011 (ρ =-0.2925, *P* = 0.0014) and a positive effect on the western chorus frog in 2012 (ρ =0.1785, *P* = 0.0349). In 2011 cloud cover was correlated with RA of the western chorus frog (χ^2 = 23.373, *P* =0.0054) and with RA of the spring peeper (χ^2 = 32.497, *P* =0.0002). In 2012 cloud cover was correlated with RA of the spring peeper (χ^2 = 24.799, *P* =0.0032). Results varied temporally.

Fragmentation

Mean distance to five nearest wetlands (Appendix 4) was negatively correlated with wood frog RA (ρ =-0.6616, *P*=0.0100), 2012 mean number of species at a site (ρ =-0.6049, *P*=0.0219), 2012 maximum number of species heard at a site (ρ =-0.7088, *P*=0.0045), and total number of species heard at a site (ρ =-0.5715, *P*=0.0328). Mean distance to 5 nearest roads (Appendix 4) was positively correlated with 2011 chorus frog RA, 2011 spring peeper RA, 2011 mean number of species heard at a site, 2011 maximum number of species heard at a site, 2012 chorus frog RA, and 2012 maximum number of species heard at a site (Table 7). Mean distance to five nearest major roads (Appendix 4) was positively correlated with 2011 spring peeper RA, almost with 2011 grey frog RA, 2011 American toad RA, 2011 mean number heard at a site, 2011 maximum number heard at a site, 2011 total number, 2012 chorus frog RA, and 2012 maximum number heard at a site (Table 6).Length of roads (Appendix 5) within a buffer (road density) was correlated with a species' RA at all five buffer distances (Table 7). Results varied temporally.

Conclusions

Some of our expectations were supported by our findings, while others were not. While we expected vernal pool species to be more abundant in areas with a shorter hydroperiod this was not true for the wood frog or the spring peeper. It is possible that the hydroperiod was too short to allow larvae to develop so they had to use more permanent areas for breeding. Green frogs, permanent pool species, did have a positive correlation with hydroperiod, as expected but bullfrogs did not. Wetland distance was negatively correlated with wood frog RA, mean number of species, maximum number of species, and total number of species heard at a site, which is what we expected given it can be a surrogate for habitat availability. As expected roads had a negative effect on multiple species along with the mean, maximum, and total number of species heard at a site. This was most prevalent for chorus frogs in which the effect was present across both years of the study. Roads fragment the habitat and may serve as dispersal barriers as well as increasing runoff and chemical pollutants (Gibbs 1998; Vos and Chardon 1998; Turtle 2000; Karraker et al 2008; Marsh et al 2008; Reeves et al 2008). Contrary to expectations no species had a preference for areas with higher canopy cover; the American toad and grey treefrog had negative associations with percent canopy cover. Species heard calling earlier in the year were negatively affected by temperature as expected, whereas those calling later, such as the American bullfrog and Northern green frog were positively associated with temperature.

Overall, all five landcover scales were equally important in terms of different species RA; each was significant two times. Floodplain forest was the most influential, being significantly correlated with RA three times across species. The spring peeper was the most affected at the landscape scale, four times it's RA was significantly correlated with a landcover type. The wood frog and spring peeper were the most affected at the microhabitat scale with their RA correlated with four variables. Wood frog RA was correlated with leaf litter depth, leaf litter percent, hydroperiod, and temperature. Spring peeper RA was correlated with percent ground cover, hydroperiod, temperature, and cloud cover. The Northern leopard frog and Blanchard's cricket frog were the least affected by microhabitat variables; none were related to RA for either species. The spring peeper was the most sensitive species being affected greatly at the landscape and local scales. We did not find a relationship between increased species richness and increased forest cover unlike others (Gibbs 1998; Kolozsvary and Swihart 1999; Houlahan et al. 2000). This may be due to the overall small number of calling species found in the area. We did find a positive correlation with ponds at the 1000 m level, which had been predicted by models (Parris 2006). RA is influenced by local, environmental, and landscape variables but to different extents depending on the species. Trends also varied temporally, possibly due to the interactions between these variables. It is also likely that some important variables were not monitored.

Scale and heterogeneity of landscape

The landscape we studied is very heterogeneous with 15 landcover types covering the region. At the 1000m scale six sites had all 15 landcover types and three sites had 14 landcover types. Even at the scale of 50m some sites had eight or nine landcover types within that small area. This provides a wide variety of habitats for anurans to reside in. This also provides edge which can be a hindrance or a help depending upon if it brings predators to the area or more food sources and waterways via ditches.We found that different spatial scales were important for different species. The northern leopard frog is typically found in open areas such as agriculture (Knutson et al. 2000; Gagne and Fahrig 2007) and negatively associated with forested areas (Guerry and Hunter Jr. 2002. However we found them to be associated with neither.

The RA of wood frogs was mostly correlated with landscape factors at larger scales: the 500 m to 1000 m distances. They were positively correlated with asphalt and ponds. Others have found them to be correlated with upland forests and swamp forests (Hecnar and M'Closkey 1998; Waldick et al. 1999; Porej et al. 2004; Gibbs et al. 2005). Wood frogs may be traveling long distances when migrating; hence areas farther away are more important.

The RA of American toads was only affected by the landscape at the largest scale, at 1000 m. Other studies found they were negatively affected by forests (Guerry and Hunter 2002; Gagne and Fahrig 2007, but see Waldick et al. 1995; Gibbs et al. 2005). At the 1000 m scale they were positively correlated with turf . The American toad prefers open areas so it is possible that what other species considered as a hindrance (turf), the American toad used for its open canopy cover.

The RA of Western chorus frogs was primarily affected at the smaller scale of 100m. There was a positive relationship between their RA and amount of residential areas, which makes sense because they are often found in areas that have been modified by humans (Conant and Collins 1991). This could be for various reasons, such as using nearby drainage ditches for breeding. They may also be edge species and do better at these junctions.

Spring peepers are known to have a positive association with forests, depend on upland habitat, and are less successful in agricultural areas (Hecnar and M'Closkey 1997; Kolozsvary and Swihart 1999; Houlahan and Findlay 2003; Gibbs et al 2005). We found the spring peeper to be positively affected at all scales except the largest, 1000m. It was associated with forests as others have found at the 50m scale (with floodplain forest) and at the 500m scale (swamp forest). At the 250m scale it was associated with both residential and shrub/scrub. Both fine-scale movements and migration dictate what landcover types are associated with the Northern spring peeper.

Grey treefrogs tend to be more abundant in forested areas and require upland habitat (Hecnar and M'Closkey 1997; Gagne and Fahrig 2007). We found no association with forested areas or any other landcover type.

Northern green frogs have been found to be associated with grasslands and open areas (Knutson et al 2000). We found the northern green frog to be positively correlated with floodplain forest at the smaller scales of 50m and 100m. It could be that they are being outcompeted in their preferred open areas at small scales and have to take what is available.

The American bullfrog can be found in both grassland habitats and deciduous forests (Trumbo et al 2012). We found no correlations among the American bullfrog and any landcover type. Because they typically don't travel far from their breeding site, it may be that the surrounding landscape is not as important to them as it is to other species.

Local and environmental variables

The RA of wood frogs was positively associated with hydroperiod, the longer the hydroperiod the greater the RA. This is in contrast to both our expectations and the findings by Rubbo and Kiesecker (2005), who found a negative association with hydroperiod. Given that wood frogs spend very little time at breeding ponds, it may be that hydroperiod is a surrogate for fuller vernal pools earlier in the season. Several of our species (American toad, American bullfrog, Northern green frog, and grey treefrog) can alter their metamorphosis time, so duration of hydroperiod is not necessarily important to them (Paton and Crouch 2002). In terms of local variables spring peepers were negatively associated with percent ground cover and positively associated with hydroperiod: the more days an area was wet the greater RA of spring peepers. There was a positive association with hydroperiod: the longer the hydroperiod the greater the RA of northern green frogs. Unlike Trumbo et al (2012), we found no relationship between RA of the American bullfrog and hydroperiod.

At a local level grey treefrogs exhibited a negative relationship with canopy cover. The wood frog, western chorus frog, and spring peeper all decreased in RA as temperature increased, which makes sense due to the fact that they call earlier in the season. The RA of the grey treefrog, American bullfrog, and Northern green frog all increased with temperature which makes sense because they call a bit later in the season. The effect of temperature on multiple species in our study follows with the fact that temperature variation affects anurans greatly; more so than other types of amphibians (Battaglin et al 2005). Higher wind speed resulted in lower RA of the American toad, but higher RA in the western chorus frog. In 2011 western chorus frogs and spring peepers were most likely to call when the cloud cover was broken. In 2012 the spring peepers were most likely to call when the cloud cover was clear. Overall some species followed expectations based on life history traits. However, local variables like canopy cover and ground cover were not as influential as anticipated. This may be due to interactions with landscape and environmental variables.

Fragmentation

We expected roads to have a negative effect on species and this is what we encountered for multiple species. Roads cause direct mortality, fragment habitat, are conduits of runoff, and foster genetic isolation. Roads had a negative affect on wood frogs with their RA reduced in areas with a higher density of roads. This is similar to findings by Veysey et al (2011) who found a negative relationship between traffic density and egg mass abundance of wood frogs. Contrary to results found by Browne et al. (2009) who found roads had a positive effect on western chorus frogs, we found roads to have a negative effect on chorus frogs in terms of road density and distance to nearest roads. Spring peepers were negatively affected by roads, with their RA being higher the further the mean distance was to the five nearest roads and RA being lower when there was a higher density of roads within 500 m. We found no correlation of grey treefrogs with roads, which is similar to findings by Trenham et al (2003), although ours did show a trend for a negative effect. Roads had a negative effect on the maximum number of species heard at a site at the 50 m, 100 m, and 500 m buffers. Relative abundances were lower when density of roads was higher. Roads did have a positive effect on one species, the Northern leopard frog, whose RAs were greater the higher the density of roads.

We expected mean distance to five nearest wetlands to be negatively correlated with species RA. This held true for wood frogs, but not for any other species. It did, however, negatively correlate with the mean, maximum, and total number of species heard at a site. It's possible that some of our species exist as metapopulations, causing a population at an individual site to be lower as multiple small populations are spread amongst the various wetlands.

In conclusion we found that scale affected species differently. The landscape was very heterogeneous with 15 landcover types in the area. Some species were more influenced by buffer sizes at small (50-100 m) scales, some were only influenced at large scales (500-1000 m) and some were affected at all scales. The same held true for local variables: they affected species differently, in part depending on their individual life histories. The mixed-disturbance landscape of this unique area likely influences the distribution and RA of several species. Management likely plays a role as well; controlled burns and mowing occurred at some of these sites and may play a part in affecting RA in these areas. Interestingly roads did not have a negative effect on all species. The construction of roads may bring resources into an area (drainage ditches, prey) that offset the negative consequences of direct mortality and runoff. It is also important to note that RA varied temporally. This is likely due to complex interactions between the landscape, local, and environmental variables and the unusually wet spring during one of our field seasons, which likely made more areas repositories for amphibians.

Amphibians are sensitive species and must be treated as such. Characteristics such as their low vagility, requirement for terrestrial and aquatic areas, permeable skin, and proneness to dessication put them at an increased risk in mixed disturbance landscapes such as the Oak Openings Region. Looking at landscape, local, and environmental variables allowed us to see patterns not expected based on life history traits alone. When managing for species it is important to include landscape and local characteristics for all species involved and not just one indicator species.

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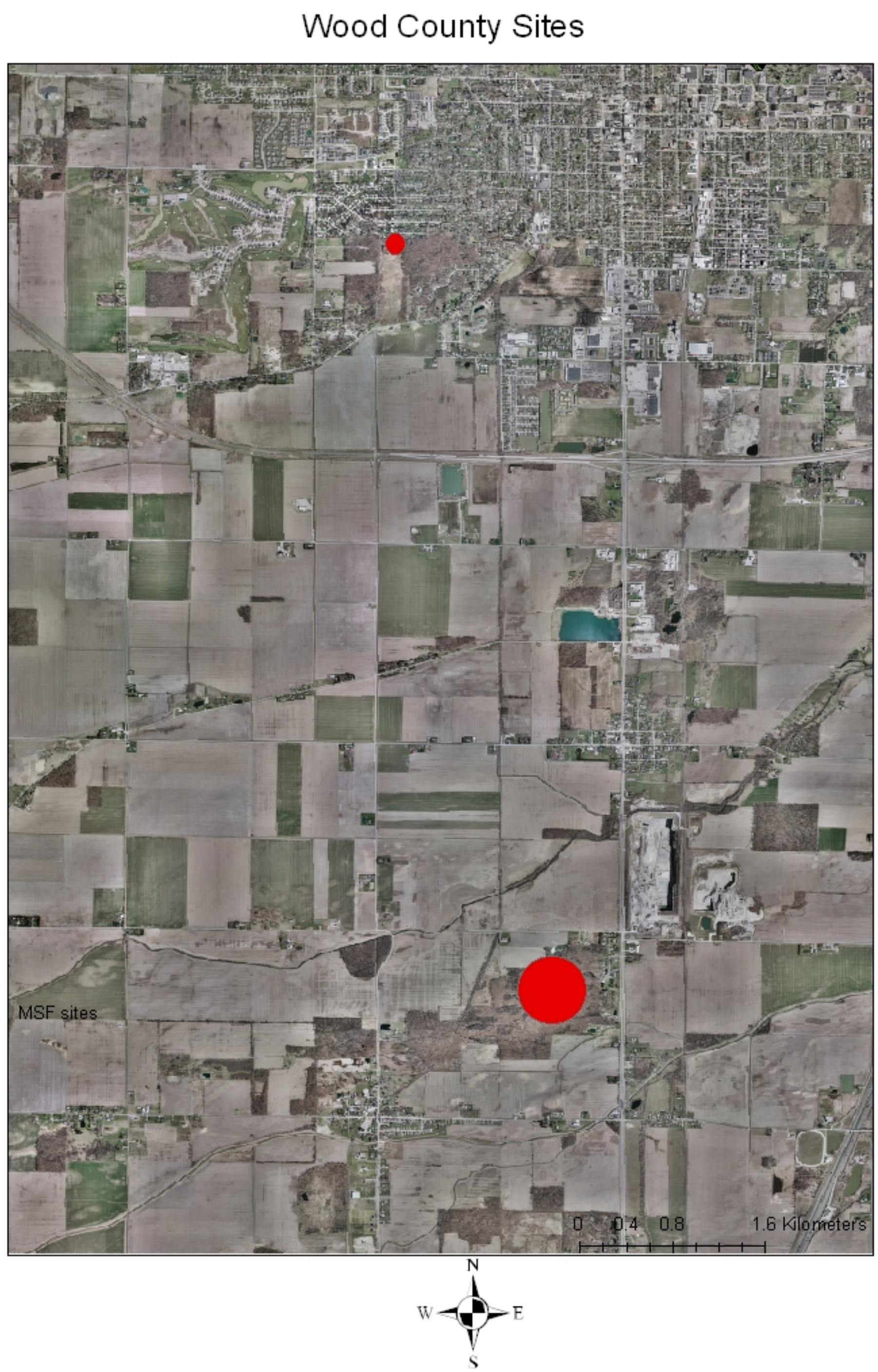

Figure 1: Map of two field sites in Wood County: Wintergarden/St. John's Nature Preserve is to the north and Steidtmann Woods is to the south, marked with red circles. Larger circle indicates larger field site.

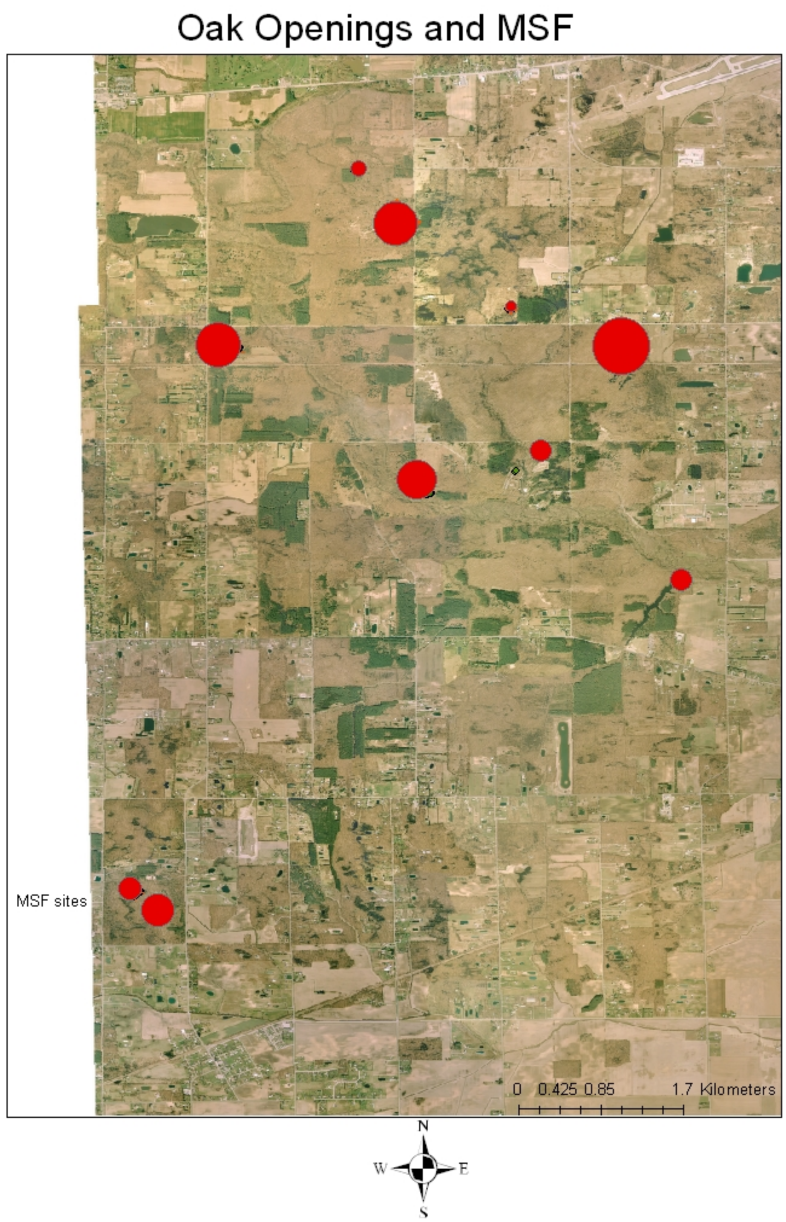

Figure 2: Eight field sites in Oak Openings Metropark to the north and two field sites in Maumee State Forest to the South, marked with red circles. Larger circles indicate larger field sites. Oak Openings sites include the following: Evergreen, GirdhamRd, Mallard, MonclovaHT, SandPit, Scout, and Yellow. Maumee State Forest sites are MSFClose and MSFFar.

Secor Metropark

Figure 3: Two field sites located in Secor Metropark, marked with red circles. Larger circle indicates larger field site. Sites are SecorFg and SecorFor.

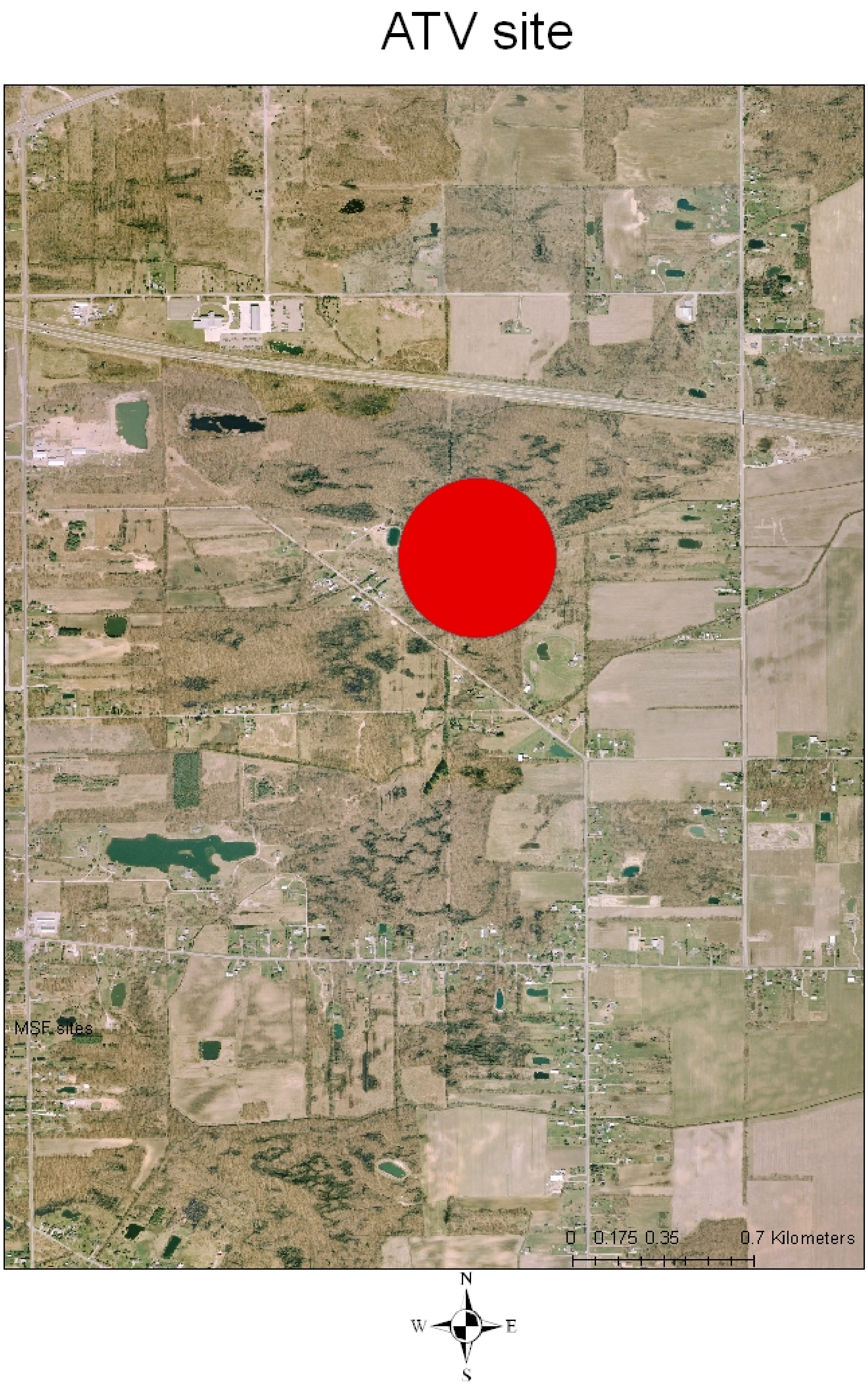

Figure 4: ATV frog calling site. Red circle indicates field site.

Table 1: Significant rho correlations between species' relative abundance (0-3 scale) and landscape type at multiple buffer distances across two years.

Species	***Year***	***Buffer (m)***	***Landscape***	***Rho***	**P**
American Toad	2011	1000	Turf	-0.7799	0.0010
Wood frog	2012	500	Asphalt	0.7239	0.0034
	2012	1000	Pond	0.8053	0.0005
Chorus frog	2012	100	Residential	-0.7409	0.0024
Spring peeper	2011	50	Floodplain	-0.7407	0.0024
	2011	250	Residential	-0.7275	0.0032
	2011	250	Shrub/scrub	0.7710	0.0012
	2011	500	Swamp	0.8022	0.0006
N. Green frog	2011	50	Floodplain forest	0.7889	0.0008
	2011	100	Floodplain forest	0.7667	0.0014

Table 2: Significant rho correlations between temperature and various anuran relative abundances (0-3 scale) across two years using calling surveys in northwest Ohio during the spring and summer of 2011 and 2012.

Variable	***Variable***	***Year***	***Rho***	***P***
Temperature (°C)	RA wood frog	2011	-0.2481	0.0070
	RA western chorus frog	2011	-0.2701	0.0032
	RA spring peeper	2011	-0.2179	0.0188
	RA grey treefrog	2011	0.2810	0.0022
	RA American bullfrog	2011	0.2836	0.0019
	RA N green frog	2011	0.4373	<0.0001
	RA spring peeper	2012	-0.2934	0.0004
	RA American bullfrog	2012	0.3165	0.0001
	RA N green frog	2012	0.2884	0.0006

Table 3: Mean percent leaf litter and mean leaf litter depth at each field site ± standard error.

Site	***Mean % Leaf Litter±SE***	***Leaf Litter Depth***	***n***
ATV	52.58±9.41	11.45±2.93	
Evergreen	0	0	
GirdhamRd	0.63±0.63	0.13±0.13	
Mallard	14.5±5.23	2.85±1.32	
Monclova	0	0	
MSFClose	100±0	25.8±4.63	
MSFFar	100±0	21±3.32	
SandPit	66.25±9.40	19.58±3.45	
Scout	41.09±8.47	3.13±0.87	
SecorFg	51.71±12.04	5.49±1.46	
SecorForest	68.8±15.90	11.56±2.83	
Steidtmann	76.33±7.01	15.8±2.64	
Wintergarden	33.33±4.08	4.13±1.47	
Yellow	60±30	20.9±2.7	

Table 4: Mean percent canopy cover and mean percent ground cover at each field site ± standard error.

Site	***% Canopy Cover***	***% Ground Cover***	***n***
ATV	52.4±11.29	87.24±8.27	
Evergreen	69.1±9.31	129.3±24.59	
GirdhamRd	14.8±5.65	113.25±8.46	
Mallard	74±6.35	48.12±6.32	
Monclova	29.2±17.59	99.31±9.01	
MSFClose	79.3±1.18	21.95±8.74	
MSFFar	81.6±3.31	71.75±6.03	
SandPit	56.9±4.39	71.45±5.22	
Scout	80.3±3.13	95.58±7.53	
SecorFg	85.1±0.98	35.87±10.43	
SecorForest	82.9±2.18	53.07±9.92	
Steidtmann	79.7±0.92	48.95±4.31	
Wintergarden	52.9±21.07	109±21.36	
Yellow	71.8±12.15	103.13±15.95	

Table 5: Shannon Diversity index for ground cover at each field site.

Site	***Shannon Diversity Index***
ATV	2.357058
Evergreen	3.053289
GirdhamRd	2.572801
Mallard	2.129392
Monclova	2.233807
MSFClose	1.847361
MSFFar	2.043318
SandPit	2.659668
Scout	2.956911
SecorFg	1.914912
SecorForest	2.019571
Steidtmann	1.992031
Wintergarden	2.851136
Yellow	2.838296

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Table 6: Significant rho correlations of mean distance to 5 nearest roads and 5 nearest major roads with various call survey variables across two years.

Variable	***Variable***	***Rho***	**P**
Mean distance to 5 nearest roads	2011 RA chorus frog	0.6307	0.0156
	2011 RA spring peeper	0.8593	<0.0001
	2011 mean number	0.5765	0.0309
	2011 max number	0.7007	0.0053
	2012 RA chorus frog	0.8352	0.0002
	2012 max number	0.5257	0.0535
Mean distance to 5 major roads	2011 RA spring peeper	0.8286	0.0003
	2011 RA grey treefrog	0.5223	0.0554
	2011 RA A toad	0.6288	0.0160
	2011 mean number	0.5655	0.0351
	2011 max number	0.7469	0.0021
	2011 total number	0.5433	0.0446
	2012 RA chorus frogs	0.4952	0.0718

Table 7: Significant rho correlations between length of roads within various buffers with different call survey variables across two years.

Variable	***Buffer***	***Variable***	***Rho***	**P**
Length of roads	50m	2012 max number	-0.5607	0.0370
	100m	2012 max number	-0.6097	0.0206
	250m	2011 RA wood frog	-0.5272	0.0527
	250m	2012 RA chorus frog	-0.5388	0.0468
	500m	2011 RA spring peeper	-0.5341	0.0492
	500m	2011 RA N leopard frog	0.5902	0.0263
	500m	2011 max number	-0.5712	0.0329
	1000m	2012 RA N leopard frog	0.6756	0.0080

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CHAPTER II

TRACKING SALAMANDER MIGRATION MOVEMENTS USING FLUORESCENT POWDER

ABSTRACT

Movement is a very important part of an amphibian's life because they must travel between two distinct habitat types: a wet area for breeding and larval development and a dry area for foraging and overwintering. Amphibians exhibit three distinct types of movement, migration by adults, dispersal by juveniles, and fine scale movements for foraging. We wanted to determine orientation, distance traveled, and path straightness in two types of salamander: *Ambystoma maculatum* Shaw (Spotted Salamander) and *Ambystoma tigrinum* Green (Tiger Salamander). We set up drift fences and pitfall traps to capture adult salamanders and measured their snout-vent length (SVL). Each salamander used was marked with a nontoxic fluorescent powder applied to their ventral surface. We returned the following night with a UV light and marked locations with powder using flags. We measured total distance moved, total displacement, number of turns ≥10°, and number of downed woody debris (DWD) crossed. Our results indicated that both species moved in a preferred direction, southwesterly. SVL did not play a role in distance for tiger salamanders but did for spotted salamanders. In addition, woody debris did not serve as obstacles to the spotted salamander; they moved straighter when they encountered them. The opposite was true for the tiger salamander, indicating that DWD may cause them to travel farther and reduce path linearity. These results suggest that the local environment has a direct influence on movement that is species-dependent.

INTRODUCTION

Movement is a very important part of an amphibian's existence because they must travel between two habitat types: wet areas for breeding and larval development and terrestrial areas for foraging and overwintering. Amphibians exhibit three distinct types of movement. One type of movement is dispersal: this is travel typically done by juveniles in which they colonize a different pond from where they were born (Semlitsch 2007). This movement is typically further than migration distances and is essential in maintaining healthy metapopulations (Marsh and Trenham 2001; Semlitsch 2007). Dispersal in juveniles tends to be more random and less directional when compared to adult migration movements (Sinsch 1997; Rittenhouse and Semlitsch 2006).

The second type of movement is migration: this is the travel of adults to ponds for breeding and their return trip back (Shoop 1968; Semlitsch 1985). It includes movement that occurs between their overwintering sites, summer refugia, and foraging areas (Lamoureux and Madison 1999; Lamoureux et al. 2002). Nighttime rainfall is a catalyst in migration (Semlitsch and Pechmann 1985; Todd and Winne 2006). Most species exhibit high breeding site fidelity and will attempt to return to a breeding pond whether it is there or not. In a study of five Appalachian mountain ponds, adult *Lithobates sylvatica* LeConte (Wood Frog) always returned to the ponds they originally reproduced in (Berven and Grudzien 1990). These movements are nonrandom with amphibians typically taking the same route to and from breeding ponds (Marty et al. 2005; Sztatecsny and Schabetsberger 2005; Rittenhouse and Semlitsch 2007). They generally travel perpendicular to the breeding pond's edge (Madison 1997). Factors that determine where salamanders travel include: soil pH, temperature, and moisture (Spotila 1972; Wyman

1988; Parmelee 1993). Spotted Salamanders travel to small burrows when migrating (Madison 1997). Multiple species do little traveling in the hot summer when they are prone to dessication (Mathis 1991; Madison 1997). In the *Plethodon cinereus* Green (Red-backed Salamander) both adults and juveniles have shown little variation in movement distances over multiple years (Ousterhout and Liebgold 2010).

The third type of movement amphibians exhibit is fine scale movement. These are short distance moves between foraging areas. They are typically done at night likely because risk of desiccation is low as is predation (Forester et al 2006). While not many studies of these extremely small movements have been done evidence indicates that for *Anaxyrus boreas boreas* Baird and Girard (Western Toad) this distance is 15 m while for *Pyxicephalus adspersus* Tschudi (African Bullfrog) it is 20 m (Long and Prepas 2012; Yetman and Ferguson 2011).

The habitat over which they travel can act as a hindrance if it is inhospitable to amphibian movements (Spieler and Linsenmair 1998; Birchfield and Deters 2005). In a study of radio-tagged amphibians, individuals avoided habitat edges and salamanders are more affected than anurans in regard to harsh habitat edges (deMaynadier and Hunter 1998; Rittenhouse and Semlitsch 2007). Given their size and low vagility, amphibians are often victims of road mortality and greatly affected by habitat alteration or fragmentation (Bowne and Bowers 2004; deMaynadier and Hunter 2000; Gibbs 1998; Sinsch 1990). In addition they are prone to desiccation because they respire through their skin. Juveniles are more affected by landscape alteration compared to adults (Patrick et al. 2008). A study investigating juvenile Wood frog movement showed that individuals avoided open canopy areas as well as harsh edges (Popescu and Hunter 2011). Juvenile Spotted salamanders preferred forested to clearcut (Patrick et al 2008). Adult and juvenile *Lithobates temporaria* Linnaeus (Common Frog) preferred meadows and hedgerows over arable lands, short-cut pastures, and road verges- this was more prevalent in juveniles (Vos et al 2007).

Amphibians vary in how far they migrate from breeding ponds when searching for overwintering sites. In general, anurans travel a greater distance than salamanders, and neither is uniformly distributed within the habitat (Rittenhouse and Semlitsch 2006). Members of the families Bufonidae and Lithobates travel significantly farther than ambystomatid salamanders (Rittenhouse and Semlitsch 2007). Species such as the *Bufo americanus* Holbrook (American Toad) have been known to migrate up to 1.5 km while species such as the Wood frog travel in excess of 300 m (Vasconcelos and Calhoun 2004; Forester et al. 2006). A study of juvenile Western toads showed juveniles moved from 1070 to 2720 m from breeding sites and used drainages for dispersal (Bull 2009).

On average, amphibians tend to move 159 m to 290 m into the terrestrial habitat surrounding breeding pools when migrating; frogs move a mean minimum distance of 205 m and mean maximum distance of 368 m, while salamanders move a mean minimum distance of 117 m and a mean maximum distance of 218 m (review Semlitsch and Bodie 2003). A study looking at juvenile *Ambystoma texanum* Matthes (Small-mouthed Salamander), Wood frogs, and American toads showed that they all oriented non-randomly when leaving the breeding pool (Walston and Mullin 2008; Homan et al 2010).

Amphibians can travel relatively long distances, but this is of little help if habitat fragmentation occurs between their breeding pond and terrestrial home. Human-made structures such as roads can also serve as barriers and negatively influence amphibian movement, presence, and abundance (Marsh et al. 2005). Roads often result in elevated mortality levels due to automobile collisions (Matos et al. 2012) and road density has been shown to decrease salamander diversity and anuran richness (Findlay et al. 2001; Porej et al. 2004). In one study salamander abundance including *Ambystoma spp.,* Red-backed salamander, and *Notophthalmus viridescens* Rafinesque (Red-spotted Newt) was 2.3 times higher at forested sites compared to roadside sites (deMaynadier and Hunter 2000). It has also been shown that natal dispersal is the most common movement type in which a road will be crossed (22.1% compared to 17.0% for migration, and 9.2% for home range movements) (deMaynadier and Hunter 2000). Other humanmade structures, such as drainage ditches, can serve as refuges and breeding sites for species such as Red-spotted newts, *Epidalea calamita* Laurenti (Natterjack Toads), *Siren intermedia* Barnes (Lesser Sirens), and the *Amphiuma tridactylum* Cuvier (3-toed Amphiuma) (Sugg et al 1988; Johnson 1997; Miaud and Sanuy 2005; Suislepp et al 2011). Research has indicated that the migratory paths of amphibians are relatively straight (Semlitsch 1981; Madison 1997). Many of these studies, however, have been based on radio telemetry data. Recently, fluorescent tracking powder has begun to be used in amphibian research to track movements. This powder has been shown to be safe for use in amphibians (Rittenhouse et al. 2006).

The goal of this study was to elucidate salamander initial migration movements using fluorescent tracking powder, a method used to track fine-scale movement. We expected that salamanders would not exhibit a uniform distribution in movement and predicted they would travel in a preferred direction. We expected that salamanders would move linearly through their environment and predicted that total distance traveled would not be significantly different from the shortest distance from the starting point to their ending point (total displacement) in order to maximize efficiency. We also predicted turns greater than 10° to be at a minimum. We expected size to have an effect on distance moved, and predicted larger individuals to travel shorter distances than smaller individuals because larger individuals are dominant and would inhabit the closer, more attractive sites first.

STUDY SITE

This study occurred at Steidtmann Woods, located in Wood County, Ohio, approximately five miles south of the Bowling Green State University (BGSU) campus. It is a 32.4ha forested area owned by BGSU. It is primarily swamp forest with two permanent pools and seven vernal pools. The main tree species of the area are *Quercus rubra* Linnaeus (Red Oak), *Quercus bicolor* Willd. (Swamp White Oak), Munchh. *Quercus palustris* (Pin Oak), *Acer saccharinum* Linnaeus (Silver Maple), *Acer rubrum* Linnaeus (Red Maple), *Quercus alba* Linnaeus (White Oak) and Lam.*Quercus velutina* (Black Oak) (Ruffer 1961). It has been invaded by *Lonicera maackii* Mill.(Bush Honeysuckle), *Elaeagnus umbellata* Thunb. (Autumn Olive), and *Alliaria petiolata* Cavara and Grande (Garlic Mustard) (Dr. Helen Michaels and Dr. Mike Plenzler, pers. comm.). It is bounded on the east by a state highway, on the west by a bike trail and agriculture, and on the north and south by forest and agriculture. There is a diverse species assemblage of amphibians at the site which include the following: Wood frog, *Pseudacris triseriata* Wied-Neuwied (Western Chorus Frog), *Lithobates pipiens* Schreber (Northern Leopard Frog), *Pseudacris crucifer* Wied-Neuwied (Northern Spring Peeper), American toad, *Lithobates catesbeiana* Holbrook (American Bullfrog), *Lithobates clamitans melanota* Rafinesque

(Northern Green Frog), *Acris crepitans blanchardi* Baird or Harper (Blanchard's Cricket Frog), Tiger salamander, Spotted salamander, and the *Ambystoma laterale* complex Hallowell (Blue Spotted complex).

METHODS

We set up one metal drift fence (27.746 m x 0.56 m, l x h) in March 2011, approximately one meter from a permanent water source previously determined to have at least three species of salamanders. Wooden stakes were placed every two to four feet for support and attached to the drift fence using zip ties. Six pitfall traps were set up at intervals along the side of the drift fence facing the water in order to capture adult salamanders as they were leaving the water after mating. We placed water and moist dirt in the traps to prevent desiccation and allow them someplace to burrow. Trapping was done over a two week period.

Traps were opened during the day and checked the following morning. We returned a few hours before sunset and measured snout-vent length (SVL) of each salamander and assigned the salamander an identification code. We then applied a fluorescent powder (Day-Glo: yellow, magenta, or orange) mixed with mineral oil to the ventral surface using a paint brush (Eggert, Peyret, and Guyetant 1999; Rittenhouse et al. 2006). This method has been shown to be safe for use in amphibians (Rittenhouse et al. 2006). Salamanders were placed on the other side of the drift fence, away from the water. Salamanders not marked were placed back in the water body they originated from.

We returned to the field site the following evening at sunset (approximately 28 hours later) to ensure the salamanders had the whole night to travel. We used a UV black light (366nm) to detect where powder marks were left and placed a flag corresponding to the salamander at each powder mark that changed direction at least 10° from the previous flag. The following day we returned to the site and measured the total distance (the distance between flags) as well as any DWD (twigs, sticks, and logs; from smallest to largest, respectively). We also measured the net displacement (shortest distance from the starting point to the last powder mark). We then calculated a path straightness index by dividing the total displacement by the total distance moved (Bell 1991; Birchfield and Deters 2005). Circular statistics were performed using Oriana v. 4 (Kovach Computing Services) software. We used Rayleigh's Uniformity test to test the null hypothesis that data were distributed in a uniform manner. Watson's U^2 test was used to test goodness-of-fit against the von Mises distribution.

JMP v.9.0 (SAS) was used for analyzing non-circular data. Spotted salamander SVL was compared to total distance moved, total displacement, # of angular changes greater than or equal to 10° and path straightness index. Analysis was done using an F-test, except for comparing path straightness index. This variable was non-normal and a Spearman's rho was performed for nonparametric data. Tiger salamander SVL was compared to total distance moved, total displacement, # of angular changes greater than or equal to ten, and path straightness index. Total distance and total displacement were log-transformed to account for normality. Log total distance and log total displacement were analyzed using an F-test. The number of angle changes greater than or equal to 10° along with path straightness index were non normal and compared to SVL using Spearman's rho test for nonparametric data.

RESULTS

Circular Data

Tiger salamanders (n=24) were tested with Watson's U^2 test and found to adhere to the Von Mises distribution, the circular equivalent of testing for a normal distribution. Spotted salamanders (n=6) did not have a sufficient sample size to check if it fit the distribution (sample size of 10 required). The mean vector for tiger salamanders was 220.952° with a mean vector length (r) of 0.452 (Fig. 1). The mean vector for spotted salamanders was 237.207° with a mean vector length (r) of 0.933 (Fig. 2). The closer the value to 1 for the mean vector length the more clustered and more closely around the mean the values are, indicating that spotted salamanders were more closely clustered than tiger salamanders. The Z statistic for the Rayleigh test indicated that both tiger salamanders and spotted salamanders were not distributed uniformly, they had a preferred direction (Z=4.893, p=0.0006; Z=5.219. p=0.0001).

Linear Data

Tiger salamanders were larger than spotted salamanders (10.0±0.2 cm v, 7.5±0.3 cm) and traveled a longer distance (19.21 ±3.72 m v. 6.15±.76 m). Total displacement was greater for tiger salamanders (16.78 ±3.34 m v. 5.50 ±1.38 m). Tiger salamanders turned ≥10° more often than spotted salamanders (5.8±1.2 v. 1.2±0.3). Spotted salamanders traveled in a straighter line than tiger salamanders (path straightness index) (0.899335±0.029490 v. 0.832321±0.030337).

Tiger salamanders (Table 1) showed no significant relationship between SVL and log total distance (F(1,22)=0.7580, p=0.3934), log total displacement (F(1,22)=0.5513, p=0.4657), number of angle changes ≥10° (Spearman's rho =-0.0141, p=0.9479), path straightness index (Spearman's rho =0.0382, p=0.8593), or obstacles crossed (Spearman's rho=0.0842, p=0.6957). There was no relationship between path straightness index and number of DWD crossed (Spearman's rho=0.1577, p=0.4618). Number of DWD crossed was significantly related to total distance moved (Spearman's rho=0.7449, p<0.0001), total displacement (Spearman's rho=0.7609, p<0.0001), and number of angle changes ≥10° (Spearman's rho=0.7031, p=0.0001). Path straightness index was related to total displacement (Spearman's rho=0.4515, p=0.0268) and nearly with total distance (Spearman's rho=0.3534, p=0.0903)

Spotted salamanders (Table 2) showed no significant effect of SVL on total displacement (F=4.1836, p=0.1103), path straightness index (Spearman's rho=-0.4058, p=0.4247), or number of DWD crossed (F(1,4)=0.5378, p=0.5040). There was a trend for total distance moved to increase with SVL (F(1,4)=5.9952, 0.0706) and for the number of angular turns ≥10° to increase with SVL (F(1,4)=5.1892, p=0.0850). There was a trend between path straightness index and number of DWD crossed (Spearman's rho=0.7650, p=0.0763). Number of DWD crossed was not related to total distance moved (F(1,4)=2.4047, p=0.1959), total displacement (F(1,4)=3.2413, p=0.1462), or number of angle changes ≥10° (F(1,4)=4.5000, p=0.1012). Path straightness was not related to total distance moved (Spearman's rho=0.0857, p=0.8717) or total displacement (Spearman's rho=0.0857, p=0.8717).

CONCLUSIONS

Migration movements are an important part of an amphibian's life cycle allowing it to travel between overwintering sites and aquatic areas for breeding. Our study was conducted at a permanent pond at Steidtmann Woods, a forested area bordered by forest, a major road, and agriculture. As expected both tiger and spotted salamanders moved linearly through their environment with path straightness indexes close to one. This was also found to be the case in other studies of spotted salamanders (Madison 1997; Rittenhouse and Semlitsch 2006). Others have found salamanders to travel the same path to and from breeding ponds in a nonrandom manner (Marty et al. 2005; Sztatecsny and Schabetsberger 2005; Rittenhouse and Semlitsch 2007). As predicted salamanders did not move uniformly. They had a preferred direction as both species moved in a southwesterly direction. This direction was likely picked because it is perpendicular to the pond edge (Madison 1997). Also, in this direction is a variety of vernal pools not offered elsewhere at the site. Interestingly, size (SVL) did not have an effect on distance moved for tiger salamanders. We predicted larger salamanders to move shorter distances but this was not the case. We believed this would occur because as potential overwintering sites became available, the larger individuals would be dominant and have their choice of sites first. We think that because we didn't follow the salamanders all the way to their overwintering sites the opportunity to see these differences did not present itself. A two-year study of red-backed salamanders found that SVL was negatively correlated with migration distance (Ousterhout and Liebgold 2010). In a study of the Eastern red-spotted newt, mass was found to have no effect on distance traveled when migrating (Roe and Grayson 2008). There was a trend in spotted salamanders for total distance moved to increase with greater SVL lengths and for total calculated displacement to increase with greater SVL lengths, although the relationship was not statistically significant. This relationship was opposite of our predictions, likely due to the same factor mentioned for tiger salamanders. There is also the possibility that, as opposed to migrating from the breeding pools, the salamanders were just venturing out in search of food or some other resource. However, if that were the case we would expect the salamanders to travel towards the pool when marked with powder, not away from it.

In tiger salamanders individuals moved farther in terms of total distance and total displacement the more DWD it encountered. We know that factors such as soil pH, temperature and moisture influence where salamanders travel (Spotila 1972; Wyman 1988; Parmelee 1993). Our results may indicate that DWD may serve as a barrier to movement and encountering debris forced tiger salamanders to travel further. This was not so for the spotted salamander. Interestingly, the more woody debris the spotted salamander encountered, the straighter the path it took, indicating these may not actually function as obstacles but rather as normal parts of the environment they are used to encountering. For spotted salamanders the longer their SVL the more turns they made that were $\geq 10°$. It may be that larger salamanders could not make as fine scale turns as smaller salamanders. Our tracking method did not follow salamanders as far as others. Studies of *Ambystoma maculatum* followed them for means of 67, 103, 64, 150, 192, and 118 m while our mean was 6.15 m (review Semlitsch and Bodie, 2003). Studies of *Ambystoma tigrinum* followed them for means of 215 and 60 m while our study followed them for 19.21 m (review Semlitsch and Bodie, 2003). However most of these studies used either radioactive tags or radiotransmitters, while our study followed each salamander over only a single night. In addition, to make sure powder wasn't washed off, we only monitored salamanders on nights when there was no rain, not the most conducive conditions for salamander movement (Semlitsch and Pechmann 1985; Todd and Winne 2006). For initial migration movements and orientation, fluorescent powder tracking is a viable technique.

Our results indicate that the local environment has a direct influence on movement and that influence is species dependent. Tiger salamanders were affected by downed woody debris but spotted salamanders were not. Coarse woody debris is important to salamanders by attracting prey, giving protection from dessication, and providing stable temperature and moisture areas (Jaeger 1980, Boddy 1983, Conant and Collins, 1998). It is important when managing salamanders to make sure enough leaf litter and woody debris is left on the ground for places for refuge. Controlled burns in these areas should be far enough away from a water body to ensure they won't alter the salamanders' habitat, at least 218 m (review Semlitsch and Bodie, 2003).

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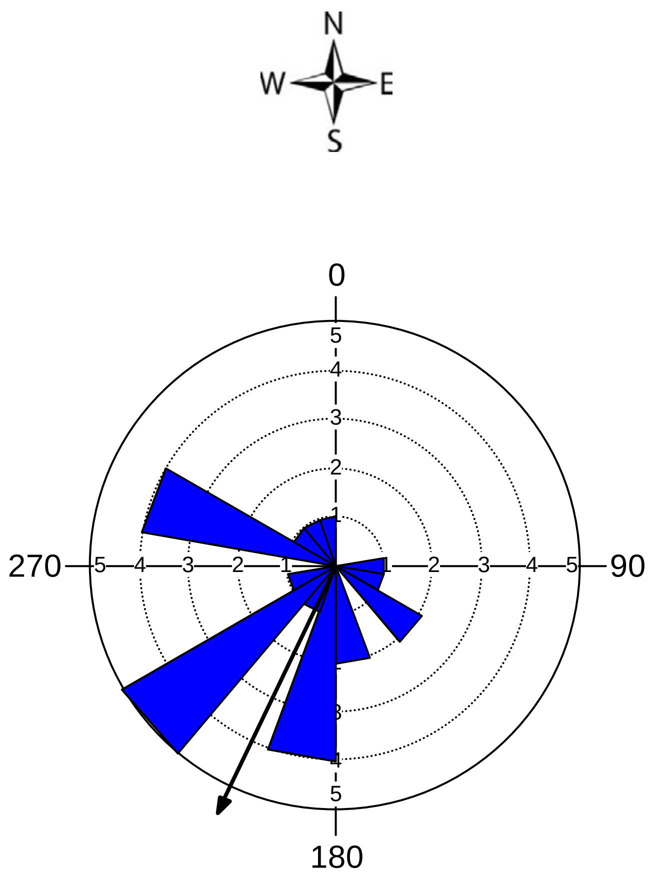

Figure 1: Mean tiger salamander vector and distribution of orientations

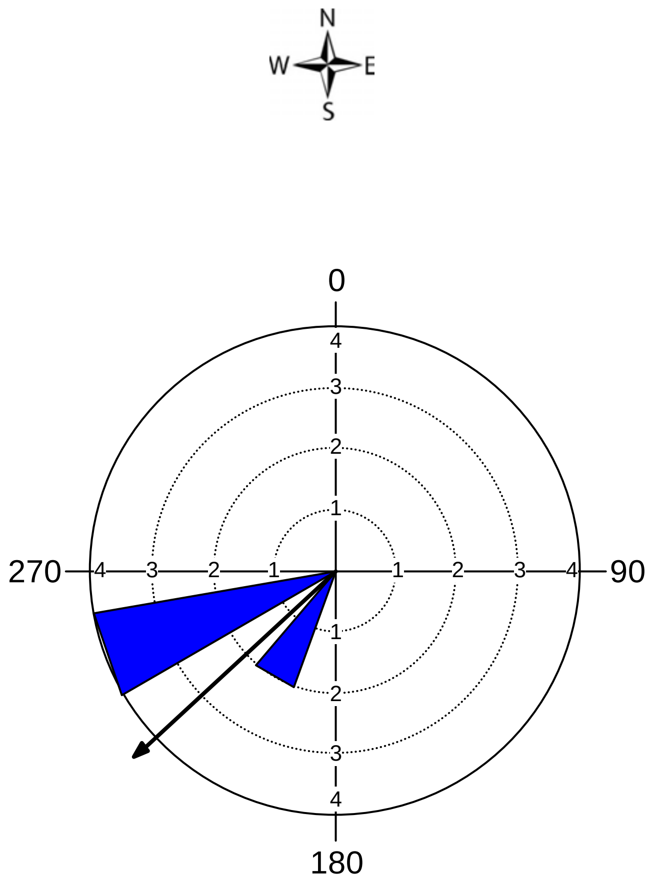

Figure 2: Mean spotted salamander vector and distribution of orientations.

Table 1: Statistical relationship between different variables of the Tiger salamander. Rho statistic used when one of the variables was non-normal. SVL=snout-vent length, # DWD (number of downed woody debris).

Variable	**Variable**	**Test statistic**	**P value**
SVL	Path straightness	Rho=0.0382	0.8593
SVL	# angle turns ≥10°	Rho=-0.0141	0.9479
SVL	Log_{10} total distance	F(1,22)=0.7580	0.3934
SVL	Log_{10} total displacement	F(1,22)=0.5513	0.4657
SVL	# DWD	Rho=0.0842	0.6957
# DWD	Log_{10} total distance	Rho=0.7449	<0.0001
# DWD	Log_{10} total displacement	Rho=0.7609	<0.0001
# DWD	# angle turns ≥10°	Rho=0.7031	0.0001
# DWD	Path straightness	Rho=0.1577	0.4618
Path straightness	Log_{10} total distance	Rho=0.3534	0.0903
Path straightness	Log_{10} total displacement	Rho=0.4515	0.0268

Table 2: Statistical relationship between different variables of the Spotted salamander. Rho statistic used when one of the variables was non-normal. SVL=snout-vent length, # DWD= number of downed woody debris.

Variable	**Variable**	**Test statistic**	**P value**
SVL	Path straightness	Rho=-0.4058	0.4247
SVL	Total distance	F(1,4)=5.9952	0.0706
SVL	Total displacement	F(1,4)=4.1836	0.1103
SVL	Angle turns ≥10°	F(1,4)=5.1892	0.0850
SVL	# DWD	F(1,4)=0.5378	0.5040
# DWD	Total distance	F(1,4)=2.4047	0.1959
# DWD	Total displacement	F(1,4)=3.2413	0.1462
# DWD	Angle turns ≥10°	F(1,4)=4.5000	0.1012
# DWD	Path straightness	Rho=0.7650	0.0763
Path straightness	Total distance	Rho=0.0857	0.8717
Path straightness	Total displacement	Rho=0.0857	0.8717

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CHAPTER III

AMPHIBIANS AND LEAF LITTER: EXPERIMENTAL AND FIELD RESULTS

ABSTRACT.— Leaf litter is a critical resource used by anurans for foraging and shelter. The goal of this study was to examine leaf litter preferences of local species in Northwest Ohio by a controlled mesocosm experiment and frog call surveys. We compared three species: the American Bullfrog (*Lithobates catesbeiana*), the Northern Green Frog (*Lithobates clamitans*), and the Northern Leopard Frog (*Lithobtes pipiens*). The American Bullfrog and Northern Green Frog rely on permanent ponds whereas the Northern Leopard Frog relies more on vernal pools. We compared use of oak vs. maple leaf litter in the controlled mesocosm experiment. There was no significant difference in leaf litter preference among the Northern Leopard Frog (n = 7), Northern Green Frog (n = 10), and American Bullfrog (n = 27) ($\chi 2$ = 1.892, df = 2, P = 0.3882). The American Bullfrog exhibited a preference for oak leaves, choosing oak 71.4% of the time. The American Bullfrog and Northern Green Frog both had a negative, though not significant, relationship with percent leaf litter indicating that species that are more dependent on permanent water bodies are less associated with leaf litter. Given the imperiled status of amphibians it's necessary to protect critical areas and make them inviting to amphibians.
Key words: American Bullfrog; Frog call surveys; Habitat; Mesocosm; Northern Green Frog; Northern Leopard Frog; Wetlands

INTRODUCTION

Adult amphibians require two distinct habitats to successfully thrive: a wet area where they can mate and reproduce and terrestrial habitat where they can overwinter and forage for food. Much is known about their aquatic needs, but studies on their terrestrial requirements are lacking. Given the imperiled status of most amphibians (IUCN 2008) it is necessary to protect critical areas and make them inviting to amphibians. Amphibians play an important role in the food web of wetlands, functioning as both predators and prey and functioning as a critical component of wetland biomass (Burton and Likens, 1975; Sparling et al., 2003; Gibbons et al., 2006). Adults are carnivorous, feeding on zooplankton, insects, and other amphibians (Sparling et al., 2003). Tadpoles can be herbivores, feeding on periphyton and phytoplankton (Wilbur, 1997), or act as filter-feeders, detrivores, or less often carnivores (Davis and Menze, 2002; Sparling et al., 2003). Tadpoles play a vital role in the recycling of nutrients and removing organisms. Amphibians are efficient at transferring energy from one trophic level to the next (Burton and Likens, 1975; Regester et al., 2006), and in temperate and tropical forests may serve as the greatest vertebrate contributors to biomass (Stebbins and Cohen, 1995; Sparling et al., 2003).

Researchers have begun performing field and controlled experimental mesocosm studies to discern what local variables are important for amphibians (Rubbo and Kiesecker, 2004; Smith and Schulte, 2008; Blomquist and Hunter, 2010; Renaldo et al., 2011). Manipulations of canopy cover and leaf litter type and depth have provided useful information for some species. These experiments suggest that: leaf litter type can affect amphibian growth (Stoler and Relyea, 2011); leaf litter type is more important than soil type when Red-Backed Salamanders (*Plethodon cinereus)* choose a substrate (Renaldo et al., 2011); reduced leaf litter depth negatively influences adult Wood Frog (*Lithobates sylvatica*) directional orientation (i.e., individuals moved towards areas with higher leaf litter levels) (Homan et al., 2010); and Wood Frogs choose microhabitats with greater canopy cover and more complex ground structure (Blomquist and Hunter, 2010).

Leaf litter is important for amphibians; both anurans and urodelans use it as a form of cover (Lynch and Myers, 1983). Its moisture content can serve as a limiting factor for some amphibian species (Wells, 2007). Leaf litter also serves as a base of the wetland food web: the decomposition of leaf litter releases carbon dioxide into the environment and recycles nutrients (Cornwell et al., 2008; Makkonen et al., 2012). Up to 99% of the dissolved organic carbon in stream ecosystems may come from leaf litter (Fisher and Likens, 1973; Stoler and Reylea, 2011). Decomposition is determined by three factors: environmental conditions along with the quality of the litter and the decomposers breaking it down the latter two, which are controlled by soil and climatic conditions (Makkonen et al., 2012). Different tree species have different leaf biomass, nutrient concentrations, and secondary compounds (Larcher, 2001; Earl et al., 2012). Species such as *Quercus* take longer to decompose and are a nutrient source for consumers longer into the season (Stoler and Reylea, 2011). Phenols that leach from leaves can be toxic to amphibians by passing through their porous skin (Kerby, 2009; Earl et al., 2012). In a mesocosm experiment comparing pure maple litter, pure oak litter, and a mixture of the two, researchers found that Wood Frogs, Jefferson Salamanders (*Ambystoma jeffersonianum*), and Spotted Salamanders (*Ambystoma maculatum*) all showed decreased survival in the pure maple treatments (Rubbo and Kiesecker, 2004). Over the past few decades there has been a shift in forest communities from oak dominated to red maple dominated (Tift and Fajvan, 1999; Bigelow and Canham, 2002; Rubbo and Kiesecker, 2004), making a comparison of these two tree species interesting and relevant, yet information on anuran preference between these species is limited.

When given the choice between deciduous and coniferous litter types both American Toad (*Bufo americanus*) metamorphs (Smith and Schulte, 2008) and Red-Backed Salamanders (Renaldo et al., 2011) preferred deciduous. American Toad metamorphs did prefer coniferous litter over bare soil (Smith and Schulte, 2008). Additionally, leaf litter can influence amphibian growth. In an experiment looking at various leaf litter combinations, Grey Treefrog (*Hyla versicolor)* tadpoles at time of metamorphosis were much larger if reared in sugar maple and eastern hemlock leaf litter than various other combinations, while those reared in a mixture of broadleaf-conifer combinations were smaller than expected (Stoler and Relyea, 2011).

Northwest Ohio is home to 10 species of anurans from three different families: Bufonidae, Hylidae, and Ranidae. These families consist of species that inhabit permanent water bodies and temporary pools. Species include the Eastern American Toad, Fowler's Toad (*Bufo fowleri*), Grey Treefrog,, Blanchard's Cricket Frog (*Acris crepitans blanchardi)*, Northern Spring Peeper (*Pseudacris crucifer)*, Western Chorus Frog (*Pseudacris triseriata)*, American Bullfrog (*Lithobates catesbeiana)*, Northern Green Frog (*Lithobates clamitans)*, Northern Leopard Frog (*Lithobates pipiens)*, and Wood Frog.

The goal of this study was to examine and compare leaf litter preferences of three anuran species of Northwest Ohio by conducting frog call surveys, measuring leaf litter, and conducting a controlled mesocosm experiment. We decided to compare three species from the family Lithobates for the mesocosm experiment: the American Bullfrog, the Northern Green Frog, and the Northern Leopard Frog. The American Bullfrog and Northern Green Frog rely on permanent ponds, whereas the Northern Leopard Frog relies more on vernal pools. The American Bullfrog is an important nuisance species that consumes smaller species as prey (Davis and Menze, 2002). Meanwhile the Northern Leopard Frog has been decreasing in numbers in Indiana and Michigan (Davis and Menze, 2002).

There are two main research questions we wanted to address. The first was whether amphibian species show a preference for areas with greater amounts of leaf litter. We predicted anurans would choose areas with higher amounts and depths of leaf litter because of the potential benefits, including places to take cover and higher abundance of insects. The second research question addressed was whether there was a difference in the choice by vernal pool versus permanent pool amphibian species for leaf litter type. We predicted permanent pool species would show a preference for *Quercus* spp. over *Acer* spp. given its longer time to decomposition and retention of nutrients.

MATERIALS AND METHODS

Study Site.—All frog call surveys were performed at sites in Wood and Lucas Counties of northwestern Ohio. Two sites were in Wood County: Steidtmann Woods (32.4 ha) owned by Bowling Green State University and Wintergarden/St. John's Nature

Preserve (41.7 ha) owned by Bowling Green Parks and Recreation. Steidtmann Woods is primarily swamp forest with two permanent water bodies and seven vernal pools. Wintergarden Nature Preserve consists of forests, prairie meadows, and a wetland area.

The remaining survey sites were in the Oak Openings Region in Lucas County, which is comprised of remnant natural ecosystems set in an urban/agricultural matrix. The Oak Openings region (467,000 ha) is extremely fragmented with 15 cover types (Schetter and Root, 2011). Two sites were at Secor Metropark (237.1 ha). This park consists of tall timber, second growth forest, sandy areas, wet lowlands, meadows, and prairies. Two sites were at Maumee State Forest (1255.7 ha). In Maumee State forest there are 15 fragmented areas with 1975 acres classified as native hardwood, 712 acres are conifer/pine plantations, 362 acres are planted hardwood, and 51 acres are wet prairie/wet sedge meadow areas. Seven sites were at Oak Openings Preserve Metropark (1523.6 ha). This diverse protected area is composed of oak savanna, oak woodland, pin oak flatwoods, sand barrens, and prairies. One site was at a former ATV site, a 24.6 ha wet prairie surrounded by forest purchased by Toledo Metroparks.

Controlled Experiment.—We set up two preassembled drift fences (24.5 m x 0.9 m and 30.5 m x 0.9 m, respectively) and one metal drift fence (10.6 m x 0.6 m) near a water source where amphibians have been caught previously. This was done at a former ATV site now owned by Toledo Metroparks and classified as a wet prairie. A total of 11 pitfall traps (five gallon buckets buried at ground level) were evenly spaced along the drift fences. During the day, bucket lids were propped up using tent stakes to prevent caught amphibians from desiccating. In addition, dirt and a wet sponge were added to provide cover and moisture, respectively. We checked the pitfall traps in the morning, at which point we covered most of the pitfall trap with its lid to prevent escape but allow adequate flow of oxygen until our return at night to place the anurans in experimental chambers.

Approximately 128.2 m from the drift fences we placed five cattle tanks (416 L each) parallel length-wise to each other (136.5 cm apart) in an open area. We added 2-2.5 cm of topsoil and 3-3.5 cm of leaf litter to each tank; half of the litter in each tank was comprised of *Acer* spp. (mixture of *Acer rubrum*, *Acer saccharinum*, and *Acer saccharum)* litter and half with *Quercus* spp. (*Quercus palustris, Quercus alba*, and *Quercus velutina*) litter (Fig. 1). Leaf litter was collected at the field site prior to the experiment and crushed into small fragments for uniformity. Tanks one, three, and five had oak litter in the right half of the tank and maple in the left, while tanks two and four had maple in the right half and oak in the left to account for a possible side bias. We placed adult anurans under PVC piping for a five minute period to allow them to acclimate to the tanks. We lightly misted tanks with water prior to a trial. After placing the frogs (American Bullfrog, Northern Green Frog, and Northern Leopard Frog) in the tank, we covered the tank with a charcoal fiberglass screen to prevent escape. Between one and four frogs of a single species were placed in the center of a cattle tank within one hour of sunset and tanks were checked for location of anurans within one hour of sunrise. Trials were conducted between 24 June 2012 and 5 August 2012 (Table 1). Choices were analyzed using a Chi Square test.

Field Study.—We conducted frog call surveys at 14 sites to assess anuran presence, relative abundance, and diversity in various wetland types of northwest Ohio. We chose both permanent water bodies and vernal pools where anurans had been heard calling previously (Karen Menard, pers. com.; K. Baczynski, pers. obs). Two of the sites were in Wood County, Ohio; the remaining 12 were in the Oak Openings Region of Lucas County, Ohio. Seven sites were small or open enough that one listening point was sufficient and seven sites had multiple listening points (>0.2 ha and closed canopy). We did not conduct surveys if there was rain or strong winds because anurans are unlikely to call during these conditions (Davis and Menze, 2002). We visited a site a minimum of 15 minutes following sunset and waited up to five minutes to hear a call. If no calls were heard by the time five minutes elapsed the site was deemed to have no calling frogs. If a call was heard, we recorded the species and estimated the relative abundance (RA). We classified the relative abundance of anurans on the following 0-3 scale (Davis and Menze, 2002; Pillsbury and Miller, 2008).

0 = no calls heard

1 = individual calls not overlapping

2 = some overlapping calls but number of individuals calling can be reliably estimated

3 = continuous chorus of calls; individual calls can't be discerned

Three minutes were spent recording species after the first call was heard. In 2012 we surveyed each site every other week from 13 March 2012 to 13 July 2012 for a total of nine visits. Sites were monitored either the same day or within three days with similar weather conditions.

We estimated percent cover of all leaf litter in 1 m x 1 m quadrats every 40 m around the edge of the water body and measured leaf litter depth at five points within the quadrat to eventually obtain a mean percent leaf litter cover and mean litter depth for each field site. Data were analyzed with a Spearman's rho correlation for nonparametric data to assess the relationship between species' distribution and abundance and the amount of leaf litter.

RESULTS

Controlled Experiment.—When we compared use of oak vs. maple leaf litter in a controlled mesocosm experiment, there was no significant difference in leaf litter preference among Northern Leopard Frogs (*n* = 7), Northern Green Frogs (*n* = 10), and American Bullfrogs (*n* = 27) (χ^2 = 1.892, df = 2, *P* = 0.3882). Overall, oak was chosen 63.6% of the time and maple was chosen 36.4% of the time. The Northern Leopard frog was the least specific in its preference choosing maple leaves 57.1% of the time and oak leaves 42.9% of the time, followed by Northern Green Frogs with 60.0% choosing oak leaves and 40.0% choosing maple leaves. The American Bullfrogs exhibited a strong preference for oak leaves, choosing them 70.4% of the time, while only choosing maple leaves 29.6% of the time (Fig. 2). The Northern Green Frog and American Bullfrog were the most similar (χ^2 = 0.358, df = 1, *P* = 0.5496) followed by the Northern Green Frog and Northern Leopard Frog (χ^2 = 0.486, df = 1 *P* = 0.4858). The American Bullfrog and Northern Leopard Frog were the least similar (χ^2 = 1.843, df = 1, *P* = 0.1747).

Field Study.—We also looked at the relationship between litter depth, percent coverage and call survey results at 14 different sites. As expected, there was a significant and positive relationship between mean litter depth and mean percent leaf litter cover (ρ = 0.9548, $p < 0.0001$).

American Bullfrog RA had a negative, though not significant, relationship with percent leaf cover (ρ = -0.1281, *P* = 0.6626) and leaf depth (ρ = -0.2303, *P* = 0.4284) as did Northern Leopard Frog RA with percent leaf litter (ρ = -0.0304, *P* = 0.9178) and leaf depth (ρ = -0.0607, P = 0.8366). Northern Green Frog RA had a negative relationship with mean leaf depth (ρ = -0.0387, P = 0.8955).

CONCLUSIONS

We predicted that permanent pool species, which breed later in the season, would show a preference for oak litter, because as a recalcitrant species, oak may provide nutrients further into the season. Only one of the two permanent pool species, the American Bullfrog, showed a preference for oak leaves (picking oak leaves 70.4% of time), while Northern Green Frogs, also a permanent pool species, showed no preference (chose oak 60.0% of the time). Northern Leopard Frogs, breeding location generalists, showed no preference (chose oak only 42.9% of the time). Both permanent water species chose oak over maple, whereas the breeding location generalist chose maple over oak. Since the Northern Leopard Frog is preyed upon by American Bullfrogs and Northern Green Frogs, it may be that oak is preferred but to avoid predation they have decided to settle for second best (ghost of competition past, Connell 1980). A second possibility is that since Northern Leopard Frog tadpoles hatch earlier than the other two species, when resources are more limited, it is more important for them to be in a water body with leaf litter that decomposes more quickly making energy more readily available so they have adapted to this situation.

Leaf litter is an important component of the wetland ecosystem, serving as a base of the food web and a protective covering for amphibians. The allochthonous energy from leaf litter input is important in most vernal pools (Wilbur, 1997) and influences macroinvertebrate production in other systems (Yanoviak, 1999; Motomure et al., 2001). Leaf litter is an important source of carbon in lentic systems (Bonner et al., 1997; Wetzel, 2001; Rubbo et al., 2006; Rubbo et al., 2008) and can account for up to 99% of total dissolved organic carbon (DOC) in lotic systems (Fisher and Likens, 1973). Recently, mesocosm experiments have begun to look at specific preferences of amphibians for leaf litter, soil type, and canopy cover. Most leaf litter experiments have compared preferences for deciduous vs. coniferous leaf litter with results yielding a preference for deciduous. One study investigating American Toad metamorphs found that they chose deciduous leaf litter over coniferous leaf litter and bare soil (Smith and Schulte, 2008). A choice experiment using the Red-backed Salamander found the salamander preferred deciduous leaves over coniferous pine needles (Renaldo et al., 2011). A study by Earl et al. (2012) showed amphibian survival to be reduced in white pine compared to red oak litter.

In order to further our understanding of preference we decided to compare two types of deciduous litter, maple and oak, both found in wetland areas. In the last few decades there has been a shift from mixed oak to red maple domination in many temperate deciduous forests of the northeastern United States (Tift and Fajvan, 1999; Bigelow and Canham, 2002; Rubbo and Kiesecker, 2004), commonly referred to as the Red Maple Paradox (Abrams, 1998). This switch in domination is due to a variety of factors including fire suppression, herbivory by deer, landscape disturbance, and defoliation by gypsy moths (Lorimer, 1984; Abrams, 1992; Abrams, 1998). This trend has occurred in Ohio where the proportion of oak and hickory has drastically decreased in relation to maple, yellow poplar, and black cherry (Kingsley and Major, 1970; Dennis and Birch, 1981; Griffith et al., 1993; Iverson et al., 2008a). In addition, deer prefer to browse on oak acorns and foliage (Bramble and Goddard, 1953) with acorns making up to 76% of a deer’s diet during a productive year (Harlow et al., 1975). Models indicate that forest composition in the eastern United States will continue to change drastically with increased climate change (Iverson et al., 2008b). Similarly, the Oak Openings Region of Northwest Ohio has historically been oak dominated, but recent changes are favoring the more adaptable and faster-growing maples. Maples outcompete oaks because of their ability to survive as both late and early successional species (Abrams, 1998). They can survive in conditions of varying nutrient, moisture, and light availability (Abrams, 1998). Although the land managers in the Oak Openings Region utilize some controlled burns in the protected areas, it has been found that low-intensity prescribed burns do not open the canopy enough to allow oak regeneration (Arthur et al., 1998; Hutchinson et al., 2005; Blankenship and Arthur, 2006).

The shift to a maple dominated system may have negative impacts on the American Bullfrog. The American Bullfrog is often seen as a nuisance species that feeds on anything small enough to swallow. By allowing the current trends to continue we may be able to reduce their population sizes if their preferred litter type is not available. The shift in canopy may not be harmful to species such as the Northern Green Frog and Northern Leopard Frog, which don’t show a preference for litter type. The decline to American bullfrogs may result in an increase in Northern Leopard frog, which are declining in number and outcompeted by the American bullfrog.

To complement the mesocosm study we compared mean litter depth and mean percent litter coverage at each of 14 sites to results from nine call surveys for Northern Green Frogs, Northern Leopard Frogs, and American Bullfrogs. Current management practices in the Oak Openings Region include prescribed burns and mowing, both of which reduce the amount of leaf litter. We found mean litter depth (0-38.4 mm) to have a positive relationship with the maximum number of species heard during a given night, but not with the total number heard over the season or the mean number of species heard at a site over the season (pers. obs.). The maximum species heard during a given night is the only one of the three aforementioned variables that looks at a single period in time, possibly indicating a temporal effect (pers. obs.). Less leaf litter favors the relative abundance of permanent pond species such as the American Bullfrog and the Northern Green Frog. Both were negatively, though not significantly, related to mean leaf depth at the field sites.

Given that leaf litter depth and percent coverage were positively associated with the maximum species heard on a given night, it appears that increased leaf litter promotes biodiversity in areas where multiple species overlap and breed at the same time (pers. obs). Since other species such as the Wood Frog have been heard calling in these areas (pers. obs.) I would recommend buffers of 400 m (Rittenhouse and Semlitsch, 2007) when mowing and conducting prescribed burns so as not to risk decreasing biodiversity.

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FIG. 1—Cattle tank mesocosm set up for trials with maple litter on the left and oak litter on the right.

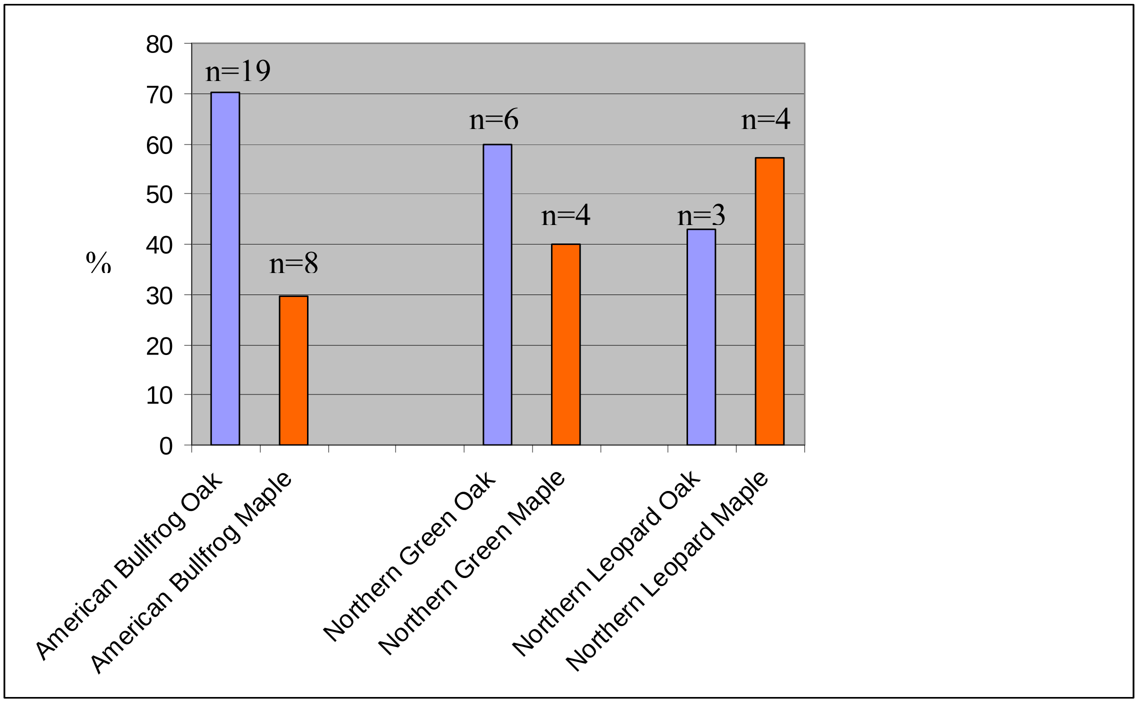

FIG. 2—Percent of occasions oak and maple litter were chosen by the American bullfrog (n = 27), northern green frog (n = 10), and leopard frog (n = 7). There was no significant difference across the species in preference (χ^2 = 1.892, df = 2, P = 0.3882).

TABLE 1—Tank number, trial number, species added, and number of individuals added to each mesocosm.

Tank Number	**Trial Number**	**Species Added**	**Number Added**
1	1	Northern green	3
1	2	Northern leopard	2
1	3	Northern green	1
1	4	Northern leopard	1
1	5	Northern green	1
1	6	Northern leopard	2
1	7	American bullfrog	3
1	8	Northern leopard	1
1	9	American bullfrog	2
1	10	American bullfrog	2
2	1	Northern leopard	1
2	2	Northern green	3
2	3	Northern green	1
2	4	Northern green	1
2	5	Northern leopard	1
2	6	American bullfrog	3
2	7	American bullfrog	2
3	1	American bullfrog	2
3	2	American bullfrog	4
3	3	American bullfrog	2
4	1	American bullfrog	2
4	2	American bullfrog	4
5	1	American bullfrog	3

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CHAPTER IV

CONCLUSIONS

The Oak Openings Region is quite diverse with 24 endangered, threatened, or of concern animal species (ODNR Division of Wildlife 2008), 143 state endangered, threatened, or potentially threatened plant species (ODNR Division of Natural Areas and Preserves 2008), five globally vulnerable plant communities (Faber-Langendoen 2001), and one federally endangered species, the Karner blue butterfly. The Oak Openings Region suffers from habitat loss and fragmentation with agriculture encroaching from the south and urbanization from the north. This makes this mixed-disturbance landscape a novel place to conduct amphibian surveys. In Chapter I I analyzed the relationships of local, landscape, and environmental variables with anuran relative abundance (RA) over multiple years. When studies of this type are done they usually don't consider all three variables, they focus on one or two. My study is also unique in that there are no published studies of amphibian RA in the Oak Openings Region.

I used frog call surveys to assess RA of the local anuran community at 15 sites in 2011 and 14 sites in 2012. ArcGIS was used to measure percent landcover, road proximity and density, and wetland proximity for each field site. I measured multiple local variables in the field including ground cover, canopy cover, leaf litter cover, leaf litter depth, and hydroperiod. Environmental variables were obtained through a local weather station. Results were inconsistent across species, meaning that different spatial scales were important for different species, likely due to differences in life history traits. These patterns did not necessarily hold over the two years of the study likely due to variable weather conditions, most noticeably the difference in spring wetness. The same held true for local and environmental variables. This information can be used by local land managers when deciding how to manage local species. It is vital that management recommendations not be based on a single species or a single season since the assemblage's requirements differ so vastly.

In Chapter II I analyzed salamander migration movements at Steidtmann Woods, an undisturbed forested area owned by Bowling Green State University. Typically movement studies utilize radiotransmitters or radio isotopes but I took a different approach. I used a fluorescent tracking powder to determine where salamanders moved.

A drift fence with six pitfall traps was set up at Steidtmann Woods at an area known to have salamanders. I trapped spotted salamanders (*Ambystoma maculatum*) and tiger salamanders (*Ambystoma tigrinum*). I measured their snout-vent length (SVL), applied powder to their dorsal surfaces, and released them on the opposite side of the drift fence. The following night I returned with a UV light and marked spots with powder using flags. Results indicated both species moved in a relatively straight line (path straightness index close to one) and traveled in a southwesterly direction with spotted salamanders being more closely clustered. Tiger salamanders showed no effect of SVL on distance moved while there was a trend in spotted salamanders for total distance moved to increase with SVL. In the tiger salamander total distance moved and total displacement was positively correlated with amount of downed woody debris crossed indicating that the local environment has a direct effect on salamander movement and that that movement is species dependent. These results can be used to better manage areas where salamanders exist and the potential obstacles that affect their movements.

In Chapter III I analyzed the effect of leaf litter on amphibians using a unique study combining both a field and controlled mesocosm experiment. For the field experiment I analyzed mean leaf litter depth and estimated percent litter coverage for each of 14 study sites. At each site, I conducted nine frog call surveys to assess RA of anuran species and correlated this with the leaf litter variables. In the controlled mescosm experiment I used three Lithobates species, the American bullfrog (*Lithobates catesbeiana*), the Northern green frog (*Lithobates clamitans melanota*), and the Northern leopard frog (*Lithobates pipiens*) to discern if they exhibited a preference for leaf litter using a cattle tank with maple litter on one side and oak litter on the other side.

Results of the field experiment indicated that none of the three focal species exhibited a significant relationship with either of the leaf litter variables measured. Results of the mesocosm experiment indicated that the American bullfrog, Northern green frog, and Northern leopard frog did not differ significantly in terms of leaf preference. However the American bullfrog did prefer oak leaves over maple leaves, choosing them 70.4%. Currently forests are undergoing a shift from an oak dominated to a maple dominated system. This may cause the American bullfrog, which is often seen as a nuisance species, to decrease in number and allow species that have been declining in number, such as the Northern leopard frog to experience a boom in population numbers. These results suggest that management actions that affect leaf litter may affect the distribution and diversity of frog species.

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Appendix 1: Class description for each of 15 different landcover classes in the Oak Openings Region.

Landcover Class	Class Description
Swamp forest	Semi-permanent to seasonally-inundated closed canopy deciduous swamps and flatwoods on poorly drained soils.
Floodplain forests	Closed to open canopy deciduous forests on poorly to moderately well drained soils within floodplains near stream channels or ditched waterways.
Upland forests	Closed canopy mesic to dry forests on moderately to well drained soils on slopes and ridges.
Conifer	Mostly monospecific plantations of *Pinus* sp. with few adventive examples.
Savanna	Open canopy stands of *Quercus velutina* and/or *Quercus alba* on well drained soils with a well developed shrub and/or herbaceous layer typically dominated by warm-season grasses and forbs.
Shrub/Scrub	Semi-permanent to seasonally-inundated shrublands on poorly drained soils.
Wet prairie	Semi-permanent to seasonally-inundated prairies on poorly drained soils. Trees nearly to entirely absent, shrubs typically sparse or absent, herbaceous layer dominated by *Carex* sp., and/or *Calamagrostis* sp.
Prairie	Mesic to dry sand prairies characterized by warm-season grasses and forbs. Trees nearly or entirely absent, shrub layer typically sparse or absent.
Sand barrens	Early successional herbaceous communities on sand blowouts and recently disturbed, well-drained soils; bare sand typically exceeds 50% of total ground cover. Trees nearly or entirely absent. Shrub layer typically sparse or absent.
Eurasian meadow	Mesic to dry cool-season grasslands and old fields dominated by Eurasian species such as *Festuca* sp., *Poa* sp., and *Bromus* sp.
Pond	Permanent excavated ponds, impoundments, and former sand mines; not associated with natural surface water drainage.
Asphalt	Areas dominated by large tracts of asphalt, parking lots, flat rooftops, and other impermeable surfaces.
Residential	Areas of closely associated residential structures, mowed lawns and shade trees; also includes roadways and maintained ditches where trees are absent.
Turf	Large areas of frequently mowed turf grasses such as cemeteries, athletic fields and golf courses; livestock pastures.
Crop	Characterized by large fields of row crops, primarily corn and soybeans.

Appendix 2a: Percent cover of each of 15 landcover types at 14 field sites within a 50m buffer

Site	Crop	Turf	Wet prairie	Resid-ential	Asphalt	Pond	Savanna	Shrub/ scrub	Swamp forest	Conifer	Upland forest	Flood plain forest	Barrens	Eurasian meadow	Prairie
ATV	0.00	22.51	2.45	6.83	0.00	0.00	0.00	0.89	19.51	0.00	16.66	27.72	0.00	0.75	2.67
Evergreen	0.00	0.00	0.00	37.91	0.00	0.00	0.00	0.00	0.00	0.00	20.08	40.46	1.54	0.00	0.00
Girdham	0.00	0.00	0.00	21.14	0.00	0.00	0.00	0.00	0.00	64.03	0.00	6.64	0.84	2.45	4.90
Mallard	0.00	0.00	0.00	6.47	0.00	0.00	0.00	0.00	35.60	8.93	3.63	45.36	0.00	0.00	0.00
MHT	0.00	0.00	0.00	10.24	0.00	0.00	9.35	0.00	2.77	28.21	0.00	12.11	5.12	4.91	27.29
MSFClo	0.00	0.00	0.00	4.83	0.00	0.00	0.00	0.00	7.47	1.06	76.02	10.61	0.00	0.00	0.00
MSFFar	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.16	0.00	87.13	3.72	0.00	0.00	0.00
Sandpits	17.34	1.93	0.00	3.70	0.00	0.00	17.13	2.46	0.00	0.00	1.84	11.34	0.00	10.62	33.64
Scout	0.00	0.00	0.00	35.21	0.00	1.83	1.14	0.00	1.83	14.65	6.05	38.39	0.00	0.00	0.90
SecFg	0.00	4.67	0.00	15.30	0.00	0.00	0.48	0.00	0.00	0.00	1.66	77.72	0.00	0.17	0.00
SecFor	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00	63.39	0.00	21.23	14.75	0.00	0.00	0.00
Steidt	0.34	1.40	0.00	6.31	0.00	0.00	0.00	0.00	13.54	0.00	25.74	52.67	0.00	0.00	0.00
Winter	0.00	9.65	0.00	1.21	0.00	0.00	0.00	0.00	3.38	0.00	5.29	32.80	0.00	33.23	14.44
Yellow	0.00	0.00	0.00	9.76	0.00	0.00	0.00	0.00	17.52	19.51	19.07	34.14	0.00	0.00	0.00

Appendix 2b: Percent cover of each of 15 landcover types at 14 field sites within a 100m buffer.

Site	Crop	Turf	Wet prairie	Resid-ential	Asphalt	Pond	Savanna	Shrub/ scrub	Swamp forest	Conifer	Upland forest	Flood plain forest	Barrens	Eurasian meadow	Prairie
ATV	0.00	17.56	2.33	6.51	0.35	0.00	1.42	0.87	18.43	0.00	22.52	25.56	0.00	2.14	2.33
Evergreen	0.00	0.39	0.00	28.43	0.00	0.00	0.00	0.00	0.00	0.00	14.86	47.56	2.07	4.61	2.07
Girdham	0.00	1.46	0.00	19.85	0.00	0.00	0.31	0.00	0.01	59.32	0.68	10.26	2.38	1.36	4.37
Mallard	0.00	1.91	0.00	10.82	0.00	0.00	0.00	0.00	24.71	22.98	8.76	30.82	0.00	0.00	0.00
MHT	0.00	0.00	0.00	3.83	0.00	0.00	5.49	0.00	8.19	37.10	0.00	15.54	1.83	6.09	21.92
MSFClo	0.00	0.00	0.00	6.93	0.00	0.00	0.00	0.00	7.16	7.14	71.34	7.43	0.00	0.00	0.00
MSFFar	0.00	0.00	0.00	5.44	0.00	0.00	0.00	0.00	10.40	0.51	79.36	4.30	0.00	0.00	0.00
Sandpits	10.67	1.28	0.00	2.74	0.00	0.00	21.82	1.83	0.04	0.00	6.18	17.27	0.00	10.90	27.28
Scout	0.00	0.33	0.00	36.51	0.00	0.86	3.25	0.00	4.66	13.46	5.31	32.49	0.63	0.80	1.72
SecFg	0.00	15.21	0.00	14.83	0.00	0.00	1.45	0.00	3.72	0.00	9.18	48.16	0.00	6.00	1.45
SecFor	0.00	0.00	0.00	5.56	0.00	0.00	0.00	0.00	41.39	1.05	26.61	25.39	0.00	0.00	0.00
Steidt	2.06	0.89	0.00	5.78	0.12	0.00	0.00	0.00	13.76	0.00	39.39	38.00	0.00	0.00	0.00
Winter	0.00	5.00	0.00	20.86	0.00	0.00	0.00	0.00	1.95	0.00	14.04	22.31	0.00	17.45	18.37
Yellow	0.00	0.00	0.00	5.66	0.00	0.00	1.06	0.00	17.25	11.32	24.61	39.53	0.00	0.51	0.06

Appendix 2c: Percent cover of each of 15 landcover types at 14 field sites within a 250m buffer.

Site	Crop	Turf	Wet prairie	Resid-ential	Asphalt	Pond	Savanna	Shrub/scrub	Swamp forest	Conifer	Upland forest	Flood plain forest	Barrens	Eurasian meadow	Prairie
ATV	1.21	10.45	0.82	10.79	0.16	0.00	1.37	0.46	19.70	0.00	24.85	24.94	0.52	2.98	1.75
Evergreen	8.05	5.76	0.00	15.05	0.00	1.20	0.00	0.42	0.83	3.43	15.83	43.07	2.00	3.57	0.80
Girdham	0.00	1.17	0.00	12.40	0.00	0.00	2.48	0.45	7.32	38.18	12.81	16.07	1.90	3.48	3.73
Mallard	0.00	2.00	0.00	14.36	0.00	0.00	0.42	0.32	13.51	28.60	17.98	20.49	0.33	1.33	0.66
MHT	0.00	0.00	0.00	7.90	0.00	0.00	6.90	0.00	4.58	31.38	12.37	13.05	0.38	6.81	16.64
MSFClo	4.50	1.66	0.00	11.58	0.00	0.00	0.00	0.76	16.32	6.16	48.80	10.21	0.00	0.01	0.00
MSFFar	0.00	0.52	0.00	11.92	0.00	0.00	0.00	0.41	16.55	8.98	49.18	12.44	0.00	0.00	0.00
Sandpits	3.95	4.28	0.00	5.11	0.00	0.00	13.76	0.87	6.16	0.26	25.48	13.02	0.26	12.68	14.16
Scout	0.00	4.49	0.00	27.46	0.00	0.25	1.74	0.00	3.19	21.40	6.89	23.47	2.18	6.23	2.69
SecFg	0.34	11.76	0.00	13.84	0.00	0.00	0.72	0.00	7.57	0.34	21.24	33.62	0.34	8.70	1.53
SecFor	0.93	2.43	0.00	10.62	0.00	0.00	0.00	0.23	18.06	1.16	31.98	29.80	0.00	4.12	0.68
Steidt	11.36	2.87	0.00	14.55	0.17	0.00	0.00	0.00	10.53	0.00	35.00	25.49	0.00	0.02	0.01
Winter	1.87	3.81	0.00	33.42	0.00	0.00	0.00	0.00	3.13	0.00	19.60	22.19	0.00	5.41	10.57
Yellow	1.29	0.24	0.00	3.00	0.00	0.00	1.29	0.18	17.08	9.35	22.46	41.30	0.00	2.29	1.51

Appendix 2d: Percent cover of each of 15 landcover types at 14 field sites within a 500m buffer.

Site	Crop	Turf	Wet prairie	Resid-ential	Asphalt	Pond	Savanna	Shrub/ scrub	Swamp forest	Conifer	Upland forest	Flood plain forest	Barrens	Eurasian meadow	Prairie
ATV	7.81	7.63	0.56	17.18	0.19	0.00	1.71	0.87	16.87	0.12	20.76	19.47	1.00	3.33	2.49
Evergreen	10.86	5.94	0.00	17.55	0.00	0.64	0.53	0.21	3.92	8.22	18.22	27.81	0.84	4.30	0.95
Girdham	0.00	1.33	0.00	8.40	0.00	0.00	3.71	0.54	16.54	22.37	23.87	14.09	1.53	4.18	3.45
Mallard	0.00	0.87	0.00	13.68	0.00	1.16	0.87	0.86	10.34	30.49	24.95	12.97	0.90	0.93	1.96
MHT	0.10	4.11	0.00	9.00	0.00	0.00	3.54	0.00	12.75	14.54	35.12	10.02	0.10	4.86	5.84
MSFClo	7.98	12.19	0.00	23.76	0.12	0.00	0.36	0.45	11.63	4.67	23.30	12.26	0.00	2.81	0.47
MSFFar	1.68	6.28	0.00	21.99	0.14	0.10	0.72	0.49	14.84	8.34	28.18	15.49	0.10	1.45	0.21
Sandpits	2.38	11.00	0.00	11.16	0.25	1.05	6.13	0.42	13.01	0.43	24.12	14.38	1.89	7.60	6.17
Scout	0.08	7.95	0.00	22.53	0.00	0.08	1.58	0.25	4.55	23.55	7.71	23.54	2.12	3.60	2.45
SecFg	1.06	11.98	0.00	8.25	0.00	0.00	1.83	0.39	6.62	0.30	17.15	39.88	0.10	9.50	2.95
SecFor	0.70	3.96	0.00	9.86	0.00	0.08	0.39	0.18	22.92	0.55	31.49	22.96	0.08	6.20	0.63
Steidt	36.07	4.69	0.06	17.27	0.28	0.00	0.00	0.06	6.36	0.06	17.86	13.94	0.77	1.22	1.35
Winter	2.48	8.05	0.00	49.63	0.00	0.00	0.10	0.00	1.54	0.00	10.32	18.55	0.48	2.71	6.13
Yellow	0.75	1.66	0.00	7.27	0.00	0.82	0.97	0.14	14.54	13.67	20.39	32.63	0.07	4.89	2.20

Appendix 2e: Percent cover of each of 15 landcover types at 14 field sites within a 1000m buffer.

Site	Crop	Turf	Wet prairie	Resid-ential	Asphalt	Pond	Savanna	Shrub/ scrub	Swamp forest	Conifer	Upland forest	Flood plain forest	Barrens	Eurasian meadow	Prairie
ATV	17.33	5.32	0.20	20.51	0.89	0.32	2.11	0.43	10.20	0.72	15.54	15.34	1.65	5.63	3.80
Evergreen	11.98	9.59	0.00	24.24	0.30	0.25	0.70	0.28	3.89	9.88	13.93	19.20	0.44	4.04	1.26
Girdham	1.81	1.18	0.00	5.43	0.00	0.00	3.67	0.28	18.04	12.86	29.69	16.54	0.74	5.73	4.04
Mallard	0.69	1.93	0.00	7.48	0.00	0.32	2.20	0.42	14.34	16.74	31.46	19.08	0.53	2.53	2.27
MHT	1.64	5.62	0.00	8.13	0.05	0.00	3.77	0.25	17.06	6.51	31.83	11.32	1.33	7.63	4.86
MSFClo	15.12	12.94	0.10	23.55	0.33	0.79	0.74	0.18	6.94	3.88	16.86	10.39	0.62	5.44	2.13
MSFFar	20.37	11.03	0.11	20.84	0.35	0.78	0.70	0.16	7.79	4.21	18.85	9.54	0.34	3.44	1.49
Sandpits	1.81	7.96	0.00	15.03	0.22	0.76	2.85	0.27	9.42	6.97	25.99	13.29	3.04	8.29	4.11
Scout	0.70	8.69	0.00	21.69	0.17	0.07	1.15	0.22	7.29	17.91	15.31	20.13	1.38	3.57	1.71
SecFg	24.39	11.77	0.10	11.25	0.42	0.04	0.71	0.23	11.48	0.21	12.18	21.26	0.17	4.58	1.21
SecFor	2.06	15.26	0.51	15.72	0.52	0.05	0.95	1.29	15.85	0.26	15.67	24.86	0.09	5.94	0.96
Steidt	55.17	3.50	0.05	13.10	2.43	0.12	0.03	0.02	2.55	0.04	13.41	7.71	0.41	0.96	0.50
Winter	5.74	11.36	0.00	62.52	1.87	0.14	0.03	0.00	0.43	0.03	2.74	8.15	0.84	3.23	2.93
Yellow	0.77	2.41	0.00	10.06	0.00	0.26	2.98	0.35	12.50	17.43	23.90	20.21	0.86	4.47	3.79

Appendix 3: Number of days wet for each of 14 survey sites.

Site	Hydroperiod (days)
ATV	119
Evergreen	49
Girdham	48
Mallard	121
MonclovaHT	23
MSFClose	87
MSFFar	87
Sandpit	122
ScoutPond	119
SecorFg	120
SecorFor	85
Steidtmann	121
Wintergarden	84
Yellow	121

Appendix 4: Mean distance to 5 nearest roads, 5 nearest major roads, and 5 nearest wetlands (± standard error) for each of 14 survey sites.

Site	5 nearest roads±SE (m)	5 major roads±SE (m)	5 wetlands±SE (m)
ATV	598.6±67.8	678.8±112.9	161.6±43.5
Evergreen	330.4±77.4	575.4±110.5	523.8±158.1
Girdham	705±202.5	908.8±255.6	609±31.7
Mallard	326.2±72.1	576.2±208.2	494.4±180.2
MonclovaHT	717.6±199.6	848.8±252.6	469.8±41.4
MSFClose	360±36.1	598.4±152	215.8±58.3
MSFFar	523.6±77.2	670.8±154.2	277.8±39
Sandpits	787.8±220.2	787.8±220.2	156.6±23.3
ScoutPond	301.6±63.8	545±167.7	415.4±90.9
SecorFrog	337.6±101.2	409.2±108.6	613.2±73.7
SecorForest	376±89.7	511.8±157.3	545±66.8
Steidtmann	413.6±19.3	588.2±116.1	266.2±25.6
Wintergarden	174.2±29.1	230.4±51.9	449±137.7
Yellow	467.6±60.7	569±115.2	541.4±86.2

Appendix 5: Length of roads (m) within 5 different buffers for each of 14 different survey sites.

Site	50m buffer (m)	100m buffer (m)	250m buffer (m)	500m buffer (m)	1000m buffer (m)
ATV	0	0	506	2206	10316
Evergreen	113	165	321	1835	7105
Girdham	234	362	671	1490	3491
Mallard	0	205	952	3087	7853
MonclovaHT	0	0	428	1519	7071
MSFClose	0	0	459	2659	5563
MSFFar	0	0	145	1707	5587
Sandpit	55	278	976	2999	8831
ScoutPond	141	249	2069	4492	8720
SecorFg	175	322	742	2331	7549
SecorFor	0	291	1145	2685	8485
Steidtmann	0	0	257	3386	9283
Wintergarden	0	93	965	4265	22502
Yellow	0	241	585	2825	8665

Appendix 6: Weather conditions for the spring of 2011 and 2012.

Variable	Spring 2011	Difference from normal	Spring 2012	Difference from normal
High temperature	92		96	
Low temperature	16		20	
Avg. max	57.5	-1.2	66.7	+7.4
Avg. min	39.3	+1.2	43.5	+5.0
Mean temperature	48.4	0	55.1	+6.2
Precipitation	15.3	+6.3	7.08	-2.17
Snowfall	4.7	-2.3	3.7	-3.7
Degree heating days	1571	-48	999	-535
Degree cooling days	71	+21	120	+67

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May 11, 2010

Dr. Karen Root
Biological Sciences
Bowling Green State University

Re: IACUC Protocol 10-008

Title:
Assessing Amphibian Species in Wetlands Along an Urban-Rural Gradient

Dear Dr. Root:

On **May 11, 2010** the above referenced protocol received **final approval** after review of the requested clarifications by the IACUC. The clarifications have been incorporated into the official copy of your protocol (see attached).

This approval expires on May 10, 2011, by which time renewal must be requested if you wish to continue work on the protocol. The Office of Research Compliance will send notification reminding you of the need for renewal in advance of that date.

Please have all members of your research team read the approved version of the protocol.

Good luck with your project.

Sincerely,

Hillary Harms
IACUC Administrator

Comments: The Metroparks of the Toledo Area permit will expire May 31, 2010. Please provide the new permit once obtained.

Summary of approved clarifications (page 2)

1. Item 12 of the application was clarified to indicate that trapping will occur from May - July. Frog call surveys occur from March - June.
2. It is not thought that the vernal pools can try up in a 24 hour period so amphibians in a trap will not desiccate due to pool drying.
3 Item 12 was clarified to clearly describe how the traps will be secured so that it is partly submerged.
4. Item 12 now indicates that the researchers will record every time they check the traps even if no amphibians are captured.
5. The application was clarified to indicate who "I" is.
6. A copy of the permits was provided.