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Variation in amount of surrounding forest habitat influences the initial orientation of juvenile amphibians emigrating from breeding ponds

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Abstract: Juvenile dispersal is important for the persistence of amphibian populations. Previous studies have observed nonrandom orientation in juvenile amphibians emigrating from breeding ponds; however, the environmental cues associated with these movements are not well understood. We examined the emigration behavior of recently metamorphosed juveniles of three pond-breeding amphibian species from three woodland ponds. We found that juvenile small-mouthed salamanders (*Ambystoma texanum* (Matthes, 1855)), American toads (*Bufo americanus* Holbrook, 1836), and wood frogs (*Rana sylvatica* LeConte, 1825) exhibited nonrandom orientation upon exiting the breeding ponds. Furthermore, we found a positive relationship between captures of juvenile small-mouthed salamanders and wood frogs and width of the surrounding forest habitat, indicating that these species are selecting areas with broader forested habitat upon exiting the breeding ponds. Our results indicate that migrating juvenile amphibians may rely on direct environmental cues because the orientation of small-mouthed salamanders and wood frogs was influenced by width of the surrounding forested habitat. These observations support previous studies suggesting that maintaining forest habitat, along at least a portion of breeding ponds, is important for the persistence of amphibian populations.

Résumé : La dispersion des jeunes est une étape essentielle pour la persistance des populations d'amphibiens. Des études antérieures ont noté l'orientation non aléatoire des jeunes amphibiens qui quittent leurs étangs de reproduction; cependant, les signaux du milieu associés à ces déplacements ne sont pas bien compris. Nous examinons dans trois étangs forestiers le comportement d'émigration de jeunes fraîchement métamorphosés de trois espèces d'amphibiens qui se reproduisent en étang. Les jeunes de la salamandre à nez court (*Ambystoma texanum* (Matthes, 1855)), du crapaud d'Amérique (*Bufo americanus* Holbrook, 1836) et de la grenouille des bois (*Rana sylvatica* LeConte, 1825) adoptent une orientation non aléatoire en quittant les étangs de reproduction. De plus, il existe une relation positive entre les captures de jeunes salamandres à nez court et de grenouilles des bois et la largeur de l'habitat forestier environnant, ce qui indique que ces espèces choisissent à leur départ des étangs de reproduction les zones possédant un habitat forestier plus étendu. Nos résultats laissent croire que les jeunes amphibiens en émigration peuvent utiliser des signaux environnementaux directs puisque l'orientation des salamandres à nez court et des grenouilles des bois est influencée par la largeur de l'habitat forestier environnant. Ces observations viennent appuyer des études antérieures qui indiquent que le maintien de l'habitat forestier en bordure d'au moins une partie des étangs de reproduction est important pour la persistance des populations d'amphibiens.

[Traduit par la Rédaction]

Introduction

The apparent declines in amphibian populations worldwide have been associated with numerous anthropogenic causes. Among the most studied causes are environmental perturbations that reduce the quality of aquatic and terres-

trial habitats (Blaustein and Kiesecker 2002; Collins and Storfer 2003). The upland habitat surrounding wetlands is important for the persistence of pond-breeding amphibians because these habitats are used as primary habitats where amphibians spend the majority of their life cycles and as potential corridors for amphibians to migrate to neighboring habitats (Semlitsch 2000; Semlitsch and Bodie 2003). Juvenile dispersal is important for maintaining amphibian metapopulation dynamics because it increases genetic diversity within metapopulations and enables recolonization of those locations that have gone extinct (Berven and Grudzien 1990; Semlitsch 2000). Thus, monitoring juvenile amphibian terrestrial movements away from breeding ponds might provide information on those habitat characteristics important to amphibian metapopulations, which could be used in future conservation efforts.

Received 22 June 2007. Accepted 12 October 2007. Published on the NRC Research Press Web site at cjz.nrc.ca on 18 February 2008.

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Habitat fragmentation typically involves the conversion of terrestrial habitats to agriculture or other human land uses, creating impediments to amphibian movements (Gibbs 1998; Rothermel 2004). Open-field habitats are usually warmer and dryer than interior forest habitats. Because of their small body sizes and permeable skin, recently metamorphosed juvenile amphibians may be vulnerable to desiccation in these anthropogenic habitats (Semlitsch 1981). In fragmented landscapes, therefore, migrating juvenile amphibians might choose to avoid traveling through open-field habitats. For example, juvenile amphibians emigrating from artificial ponds avoided old fields and other open habitats and selected forest interior habitats (deMaynadier and Hunter 1999; Rothermel and Semlitsch 2002).

Previous studies have demonstrated that recently metamorphosed amphibians orient nonrandomly when emigrating from their breeding ponds (Sinsch 1990; Rothermel 2004; Vasconcelos and Calhoun 2004; Patrick et al. 2007). This behavior might indicate selection for certain terrestrial habitats. However, the environmental cues associated with these movements are not well understood (Dodd and Cade 1998; Rothermel 2004; but see Jenkins et al. 2006). Identifying those cues associated with amphibian orientation behavior will be a critical aspect of amphibian conservation.

As a component of a larger study examining amphibian community dynamics (Walston and Mullin 2007), we investigated amphibian orientation behavior in a fragmented landscape in east-central Illinois, USA. Specifically, we monitored the movement patterns of recently metamorphosed small-mouthed salamanders (*Ambystoma texanum* (Matthes, 1855)), American toads (*Bufo americanus* Holbrook, 1836), and wood frogs (*Rana sylvatica* LeConte, 1825) from ponds surrounded by forest and forest-edge habitats. Our objectives for this study were to determine (i) if juveniles of each species exhibited random orientation upon exiting the breeding ponds and (ii) if juvenile orientation is associated with characteristics of the forested upland habitat surrounding the pond.

Materials and methods

Study area

We conducted our study at Warbler Woods Nature Preserve (WWNP; 39°26'N, 88°8'W), Coles County, Illinois, USA. This site contains approximately 81.5 ha of oak-hickory hardwood forests and old-field upland habitat (primarily goldenrod *Solidago* L.), which is managed by the Illinois Department of Natural Resources Nature Preserves Commission. Woodland ponds present within WWNP ranged between 0.05 and 0.10 ha in size (Fig. 1). Vegetation in and immediately peripheral to all ponds included green algae, common duckweed (*Lemna minor* L.), Dudley's rush (*Juncus dudleyi* Wieg.), stinging nettle (*Urtica dioica* L.), as well as a few other aquatic macrophytes. There is considerable variation in the upland habitat surrounding the WWNP ponds. Along some sides of each pond, the terrestrial forested buffer extends beyond 150 m. However, a portion of each pond borders much less forest habitat, where the pond's bank lies within 10 m from disturbed landcover types such as old-field upland areas, row crop agriculture, residential property, or a road (Fig. 1).

We installed drift fences around three woodland ponds at a distance of 2–3 m from each pond's high water mark. The drift fence consisted of black silt fence material, approximately 45 cm high with 10 cm buried beneath the soil surface. Because of flooding and excessive vegetation surrounding the ponds, we were not able to completely encircle each pond with the drift fence. In total, the installed drift fences encircled 90%, 85%, and 95% of ponds 1, 2, 3, respectively (Fig. 1). At each pond, pitfall traps (3 L plastic tubs, 20 cm deep) were placed along both sides of the fence at 7.5 m intervals. Small holes (<2 mm diameter) were drilled in the bottom of the pitfalls to allow water to drain and prevent captured organisms from drowning. Pitfall traps were checked at least once every 48 h during the amphibian activity period (early February through early December) between the years 2003 and 2005. We placed lids on the traps during periods of inactivity to prevent mortality of nontarget organisms.

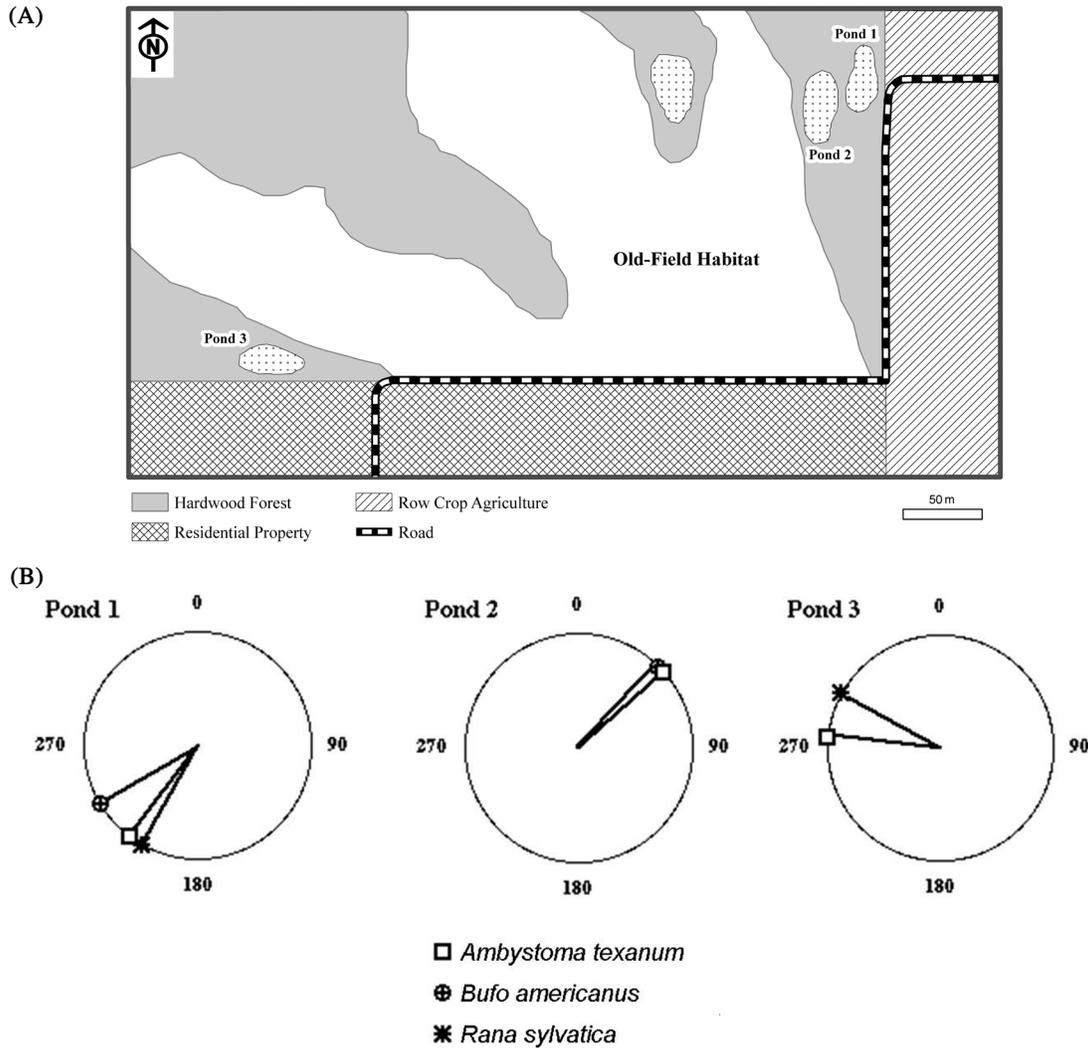
We measured the snout-vent length (SVL; ± 1 mm) and determined the reproductive class (recent metamorph (juvenile), subadult, or adult) of all captured amphibians. Only recently metamorphosed juveniles (those captured in the same season in which metamorphosis occurred) were included in this study. We marked all captured amphibians to a year-by-pond-specific cohort using a pattern of toe clipping. All recaptures were omitted from analyses.

At each of the three study ponds, we determined the azimuth from the center of the pond to the surrounding pitfall traps. We also measured two characteristics of the upland habitat at each trap location. Using a densiometer, we measured the amount of canopy cover directly above each pitfall trap. Because of the size of the ponds, canopy cover was not complete over any one of the ponds. Following the azimuth from the center of the pond, we also measured the width of the forest buffer habitat at each pitfall trap as the straight-line distances extending from the pond's edge, through each trap location, to the far edge of the forested habitat. At few trap locations (<20% of the pitfall traps), forest buffer width exceeded 150 m and was associated with much larger contiguous forest habitat (Fig. 1). In these cases, we felt it impractical to measure forest buffer width beyond 150 m. Therefore, we measured forest buffer width continuously to a maximum of 150 m.

Data analysis

Statistical analyses were conducted only for those species where $n \geq 30$ within a pond. Even though the ponds were elliptically shaped, circular statistics were used to analyze initial orientation of recently metamorphosed amphibians. Because of the departure of circularity, however, we only discuss general directions of amphibian initial orientation rather than exact angles. We assumed that the drift-fence coverage at each pond was sufficient to detect directional movements. We calculated the mean (1 SD) angle of departure for each species at each pond and used Rayleigh's test ($\alpha = 0.05$) to determine if the directions of movements deviated from a random circular distribution (Batschelet 1981). Preliminary analyses indicated that within-pond juvenile orientation was not noticeably different across years (multiple Rayleigh's tests); therefore, we pooled the data across years prior to analysis. Circular statistics and Rayleigh's tests were

Fig. 1. (A) A portion of Warbler Woods Nature Preserve, Coles County, Illinois, with the locations of the woodland ponds analyzed in this study. (B) Mean vector of orientation for recently metamorphosed small-mouthed salamanders (*Ambystoma texanum*), American toads (*Bufo americanus*), and wood frogs (*Rana sylvatica*) from the three woodland ponds. At each pond, only those species where $n \geq 30$ were included in the analysis.



calculated using Oriana software (version 2.00; Kovach Computing Services, Pentraeth, Wales, UK).

For each species, we determined the proportion of individuals captured at each pitfall trap to the total number of individuals captured at the pond. We transformed the forest buffer width and canopy cover measurements using percentile ranks to standardize these measurements across all ponds. Forest width and canopy cover were weakly correlated (Pearson's correlation, $r = 0.37$, $P = 0.001$). Preliminary analyses indicated that there were no interactions between pond type and habitat variables on captures of each species ($P \geq 0.31$ for all species). Therefore, we pooled captures across only those ponds at which orientation analyses were performed. Relative captures of each species could not be normalized. To associate captures with the measured habitat variables, we used a generalized estimating equations (GEE) analysis (PROC GENMOD; SAS Institute Inc. 1999). GEE analysis is an extension of generalized linear models that can analyze correlated data (e.g., repeated measurements at a pitfall trap) with non-normal distributions

(SAS Institute Inc. 1999). For this analysis we used a gamma distribution with a log link. The scale parameter was estimated by the degrees of freedom divided by the deviance (DSCALE). The GEE analysis was conducted using SAS[®] version 9.1 ($\alpha = 0.05$).

Results

During this study, we captured a total of 4615 recently metamorphosed juvenile amphibians from all three species at ponds 1, 2, and 3 at WWNP. Juveniles of each species exhibited nonrandom orientation when exiting the WWNP ponds (Table 1). At pond 1, all species exited along the southwestern side of the pond. At pond 2, only juvenile small-mouthed salamanders and American toads were captured in sufficient numbers. Both species exited pond 2 along the northeastern portion of the pond. At pond 3, only juvenile small-mouthed salamanders and wood frogs were captured in sufficient numbers. Both of these species exited pond 3 along the western side of the pond. In all cases,

Table 1. Orientation of recently metamorphosed small-mouthed salamanders (*Ambystoma texanum*), American toads (*Bufo americanus*), and wood frogs (*Rana sylvatica*) emigrating from three breeding ponds at Warbler Woods Nature Preserve (WWNP), Coles County, Illinois.

Pond	Species	<i>n</i>	Mean angle (°)	Circular SD (°)	<i>Z</i> _{calc}	<i>P</i>
1	<i>A. texanum</i>	262	216.7	41.4	103.8	<0.001
	<i>B. americanus</i>	868	238.9	65.1	475.0	<0.001
	<i>R. sylvatica</i>	273	209.5	34.2	191.2	<0.001
2	<i>A. texanum</i>	436	48.2	47.6	111.6	<0.001
	<i>B. americanus</i>	2537	44.9	66.9	425.1	<0.001
	<i>R. sylvatica</i>	13	—	—	—	—
3	<i>A. texanum</i>	150	275.1	51.6	47.2	<0.001
	<i>B. americanus</i>	15	—	—	—	—
	<i>R. sylvatica</i>	61	299.7	41.1	12.7	<0.001

Note: At each pond, the number of individuals captured for each species (*n*) is given. If *n* ≥ 30, circular statistics (mean angle and circular SD) are determined with results of the Rayleigh test.

juvenile emigration appeared to be oriented toward areas with >100 m of forested habitat (Fig. 1).

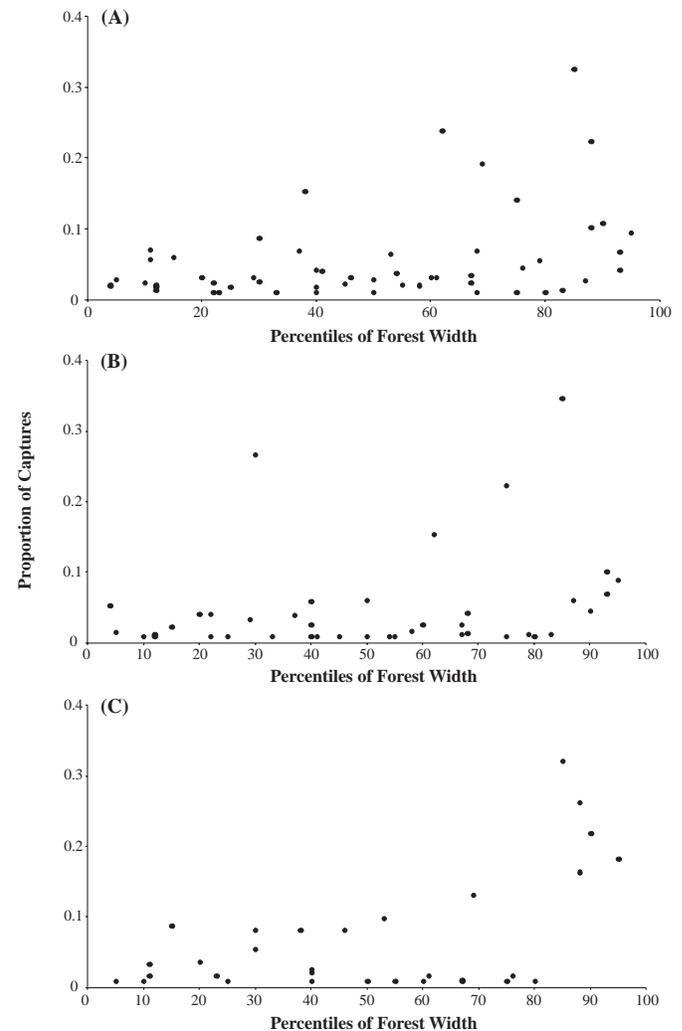
There was a positive relationship between the width of the forested habitat and the captures of juvenile small-mouthed salamanders ($\chi^2_{[1,51]} = 12.76$, $P < 0.001$) and wood frogs ($\chi^2_{[1,28]} = 7.98$, $P = 0.005$), whereas forest buffer habitat had only a marginal effect on captures of juvenile American toads ($\chi^2_{[1,39]} = 3.21$, $P = 0.073$; Fig. 2). Canopy cover was not associated with captures of any of the three species (small-mouthed salamanders: $\chi^2_{[1,51]} = 0.24$, $P = 0.627$; American toads: $\chi^2_{[1,39]} = 0.34$, $P = 0.560$; wood frogs: $\chi^2_{[1,28]} = 0.53$, $P = 0.467$).

Discussion

We observed nonrandom orientation behavior of recently metamorphosed juvenile amphibians emigrating from three breeding ponds. Nonrandom orientation behavior in migrating amphibians is well documented (e.g., Dodd and Cade 1998; Vasconcelos and Calhoun 2004; Patrick et al. 2007). However, few studies have associated amphibian orientation with specific habitat characteristics (but see Jenkins et al. 2006). In our study, the mean angle of orientation of each species appeared to be directed towards areas with greater forest habitat surrounding each pond (Fig. 1, Table 1). Furthermore, we found that the relative captures of juvenile small-mouthed salamanders and wood frogs were positively associated with wider forested habitat (Fig. 2), indicating that dispersing juveniles of these species select areas with greater forested habitat rather than forest-edge habitat. These results are based upon observations of initial orientation of juvenile amphibians <3 m from the pond's edge; we cannot rule out the possibility that amphibians may shift their orientation once they have moved farther away from the pond's edge.

Although we detected a relationship between the amount of forest habitat and relative capture rates of juvenile small-mouthed salamanders and wood frogs, this relationship was not supported for juvenile American toads despite the similar orientation patterns exhibited by all three species. This result might be explained by the variation around the mean angle of orientation for each species. For ponds at which American toads were analyzed, the circular SD for

Fig. 2. Relationship between captures of recently metamorphosed (A) small-mouthed salamanders (*Ambystoma texanum*), (B) American toads (*Bufo americanus*), and (C) wood frogs (*Rana sylvatica*) with percentiles of the forest habitat width surrounding the breeding ponds at Warbler Woods Nature Preserve (WWNP), Coles County, Illinois. For each species, only ponds at which *n* ≥ 30 were analyzed.



American toad orientation was greater than that for small-mouthed salamanders and wood frogs (Table 1). The widely dispersed movements by juvenile American toads may have obscured a relationship between orientation and amount of forested habitat. Some ambystomatid salamanders and wood frogs are considered forest-sensitive species (Berven and Grudzien 1990), whereas American toads are generalists and might be more tolerant of disturbed habitats such as old-field habitats (Kolozsvary and Swihart 1999).

We found that canopy cover was not associated with captures of any of the three species, which contrasts with previous studies that have examined target-oriented movements in amphibians. For instance, Jenkins et al. (2006) found that post-metamorphic juvenile marbled salamanders (*Ambystoma opacum* (Gravenhorst, 1807)) initially oriented towards areas with greater canopy cover. In our study, the relative variability in canopy cover at each pond was low (coefficient of variation <20% at all ponds) owing to all pitfall traps being installed in forest or forest-edge habitat. Our results indicate that, in fragmented landscapes where forest-edge habitat represents a considerable portion of the available habitat surrounding breeding ponds, canopy cover immediately surrounding the breeding pond may be a poor indication of initial juvenile amphibian orientation.

The association between amphibian orientation and characteristics of the terrestrial habitat surrounding breeding ponds has been well studied and is often debated. In a study at nine breeding ponds, Jenkins et al. (2006) found no hard-wired directional bias in the movements of juvenile marbled salamanders, suggesting the influence of direct environmental cues (such as canopy cover) in amphibian orientation behavior. Patrick et al. (2007), however, reported that the directional movements of translocated juvenile wood frogs were similar to the directional movements exhibited by juveniles from the natal pond, suggesting that migrating amphibians may rely on indirect environmental cues rather than direct cues. Our results indicate that dispersing juvenile amphibians may rely on direct environmental cues, as we found that the initial orientation of juvenile amphibians differed among ponds and was influenced by width of the surrounding forest habitat. The mechanisms by which juvenile amphibians are able to detect areas with wider forested habitat are unclear, but previous studies have implicated that olfactory cues in the substrate may direct amphibian movements (McGregor and Teska 1989; Rittenhouse et al. 2004). Furthermore, microhabitat conditions within interior forest habitat might be more hospitable to amphibians, where soil moisture is greater and temperatures are less variable than forest-edge habitats (Corn and Bury 1989). While we did not investigate olfactory cues or microhabitat variables in this study, it is possible that at least one of these mechanisms was involved in the orientation of amphibians in our study.

We did not detect directed movements by any amphibian species towards any of the disturbed landcover types (Fig. 1). These landcover types impede amphibian movements because they are possible sources of amphibian mortality, or induce higher levels of physiological stress (Gibbs 1998; Rothermel 2004). The apparent avoidance of these disturbed landcover types by the amphibians in this study implies that forest fragmentation can alter the initial orientation behavior of juvenile amphibians emigrating from breeding ponds.

The importance of the terrestrial habitat surrounding breeding ponds for local amphibian populations is widely recognized within the scientific community (Semlitsch 2000; Semlitsch and Bodie 2003). We suggest that maintaining broad forested habitats, at least along some portion of the pond, is necessary to ensure the survival of juvenile amphibians. However, the protection of such habitat should be given specific consideration across all amphibian life stages. Consequently, studies focused on elucidating the target-oriented movements of juvenile amphibians, as well as adults, will allow us to develop long-term conservation strategies.

Acknowledgements

We thank L.B. Hunt and the Illinois Department of Natural Resources (IDNR) Nature Preserves Commission for access to this study site. Fieldwork was conducted under the authorization of IDNR scientific collection permit No. NH04-0946 and an Eastern Illinois University (EIU) Institutional Animal Care and Use Protocol (No. 04-008). Funding for this research was provided by the IDNR Wildlife Preservation Fund, The Graduate School at EIU, and the EIU College of Sciences. We thank those people who assisted with fieldwork: E. Casey, D. Cox, M. Fincel, J. Florey, C. Foster, S. Klueh, M. Sikich, M. Walston, and especially L.M. Gross and J.B. Towey. We also appreciate the constructive comments on earlier drafts of the manuscript from members of the EIU Herpetology Laboratory and two anonymous reviewers.

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