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SEASONAL TERRESTRIAL MICROHABITAT USE BY GRAY TREEFROGS (*HYLA VERSICOLOR*) IN MISSOURI OAK-HICKORY FORESTS

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ABSTRACT: It is important to identify and understand the critical habitat components of organisms inhabiting landscapes that are increasingly altered by human activities to adequately predict the effects of habitat alteration on natural populations. Our study identifies terrestrial habitats that are important to the gray treefrog (*Hyla versicolor*) during the nonbreeding season using radiotracking and mark-recapture/release of individuals captured in artificial arboreal refugia. High humidity and rainfall were associated with decreased captures in artificial refugia, while high ambient air temperatures were correlated with increases in the number of treefrog captures. Refugia placed in small trees recorded more small individuals than those in larger trees, and refugia in white oaks (*Quercus alba*) contained females more frequently than expected at random. We found that space-use estimates encompassing foraging and overwintering locations of gray treefrogs were relatively small and typically included only a few adjacent trees during the nonbreeding season. We discuss the physical and biological aspects of habitat patches that may be important in determining the persistence of gray treefrog populations.

Key words: Amphibian; Diurnal refugia; Foraging site; *Hyla*; Land-use; Microhabitat; Overwintering location; Radiotelemetry; Tree cavity; Treefrog

IT HAS LONG been recognized that the biphasic lifestyle of amphibians renders their populations susceptible to changes in both aquatic and terrestrial habitats (Wilbur, 1980). For pond-breeding amphibians, aquatic habitat is most certainly important for the persistence of populations, and a great deal of research has been aimed towards understanding the factors involved in pre-metamorphic population regulation. As a result, we have a good understanding of how aspects of aquatic habitat such as hydroperiod (Pechmann et al., 1989; Phillips et al., 2002; Skelly, 1996), canopy cover (Skelly et al., 2002),

pathogens (Kiesecker and Skelly, 2001) and predator/prey dynamics (VanBuskirk and Smith, 1991; Wilbur et al., 1983) interact with conspecific density to affect growth and survival of amphibian larvae (Wilbur, 1980, 1987).

Conversely, investigations of post-metamorphic population regulation have been hindered by the difficulty in capturing and observing individuals at distances from breeding sites (Pechmann, 1995; Trenham and Shaffer, 2005), despite the fact that terrestrial habitat adjacent to aquatic breeding sites is used extensively by adults and juveniles (e.g., Semlitsch, 2000; Semlitsch and Bodie, 2003). However, conservation concerns regarding amphibian population declines (e.g., Houlihan et al., 2000; Stuart et al., 2004) have exacerbated the need for studies of pond-breeding amphibians in upland habitats (e.g.,

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Marsh and Trenham, 2001; Semlitsch, 1998; Storfer, 2003). Moreover, alteration of post-metamorphic vital rates (i.e., growth, survival, fecundity) may play a much larger role in population persistence than previously believed (Biek et al., 2002; Harper and Semlitsch, 2007; Taylor and Scott, 1997; Vonesh and de la Cruz, 2002). Recent studies of *Ambystoma* in upland habitats have shown that the availability of appropriate terrestrial habitats affects both migratory movements and survival (Rittenhouse and Semlitsch, 2006; Rothermel and Semlitsch, 2006; Trenham and Shaffer, 2005). Similarly, variation in abiotic factors such as rainfall and temperature also affects movement and survival in terrestrial habitats for *Rana* (e.g., Berven, 1990) and *Ambystoma* (e.g., Semlitsch, 1985; Trenham et al., 2000). Therefore, understanding upland habitat requirements adjacent to breeding sites could reveal mechanisms affecting local population persistence through interference with post-metamorphic activities of pond-breeding amphibians.

The effects of terrestrial habitat alteration on amphibian populations cannot be adequately predicted until we gain an enhanced understanding of the habitat features utilized by amphibians, the factors that influence their microhabitat choices (e.g., Renken et al., 2004), and the extent of terrestrial movements (deMaynadier and Hunter, 1995; Lemckert, 2004; Smith and Green, 2005). Therefore, we investigated the terrestrial behavior of the gray treefrog (*Hyla versicolor*) during the nonbreeding season. We used both mark-release/recapture techniques and radiotracking to: (1) monitor the relationship between weather variables and frequency of captures within artificial refugia and on daily microhabitat locations of individuals implanted with radiotransmitters, (2) test the correlation of tree characteristics, such as species and diameter, on use of artificial refugia, and (3) report on the extent of foraging grounds and the microhabitat in which treefrogs overwinter. These aspects of terrestrial habitat-use provide important data regarding the potential impacts of habitat alteration on arboreal amphibians and can be used to implement land-use strategies that incorporate daily and seasonal amphibian activities to minimize negative effects on both individuals and populations.

METHODS

Study Species

Gray treefrogs are common arboreal anurans throughout their range in the Eastern/Central regions of the United States and into Canada (Wright and Wright, 1949). Gray treefrogs breed from late April through July in Missouri and are the only arboreal anurans present at our study sites (J. R. Johnson, unpublished data). During the breeding season, males actively call at ponds for several consecutive nights before leaving the chorus, presumably to forage and subsequently return (Johnson et al., 2007). Females, however, are present at breeding ponds only long enough to mate and deposit eggs, usually during a single night, once or twice per breeding season (Johnson et al., 2007; Ritke et al., 1990). The specific habits of gray treefrogs are largely unknown when absent from breeding sites during either the breeding or nonbreeding seasons; however, they are generally believed to require some amount of forested habitat adjacent to breeding sites where they reside diurnally in tree cavities (McComb and Noble, 1981; Ritke and Babb, 1991). Furthermore, anecdotal reports have indicated that treefrogs may overwinter in tree cavities or on the ground (Burkholder, 1998) and that tree cavities are potentially used as foraging locations (Park et al., 1950).

Study Sites

We captured gray treefrogs in artificial arboreal refugia (see Johnson, 2005 for specifications) at the University of Missouri's Baskett Wildlife Research Area (BWRA) in southern Boone County, Missouri, U.S.A. Artificial refugia consisted of 60-cm-long sections of 3.8-cm-diameter black plastic pipe attached via bungee cord with an open end at a height of 3 m. Refugia were sealed at the bottom, and designed to retain rainwater at a depth of 15 cm. The upper opening was unobstructed, and treefrogs were not restrained within refugia. We placed refugia on large diameter deciduous trees (i.e., diameter at breast height [DBH] >10 cm) and arranged them into seven paired transects (i.e., a single pipe on each of two adjacent trees approximately 2–3 m apart at each distance) radiating from three closely associated breeding sites (2 transects at each of

two ponds and 3 transects at the third) into secondary growth oak/hickory (*Quercus* spp./*Carya* spp.) forest with sugar maple (*Acer saccharum*) understory.

Sugar maples were the most abundant tree species at our study sites ($n = 566/\text{ha}$) followed by white oaks (*Q. alba* and *Q. prinus* [$n = 264/\text{ha}$]), red oaks (*Q. rubra* and *Q. velutina* [$n = 84/\text{ha}$]), and mockernut hickory (*C. tomentosa* [$n = 78/\text{ha}$]). Basal areas of these four most common tree categories were 2.1, 11.3, 4.4, and 0.5 m², respectively. Sugar maples represented 23% of the trees with refugia attached, while white oaks comprised 20% and red oaks represented 17% of the total. Trees in which artificial refugia were placed ($n = 98$) were selected based on their position at nodes (1, 5, 15, 35, 60, 100, and 200 m) along transects extending from the breeding pond edges (Johnson et al., 2007). Sampling locations were paired to increase treefrog captures while not appreciably increasing the amount of time required to monitor all artificial refugia and to provide data regarding movements of individuals between refugia in close proximity.

Monitoring and Radiotracking

We monitored refugia for the presence of treefrogs every other day during daylight hours for the duration of treefrog activity periods (April through November) beginning in September 2002 and ending in May 2005. If present, treefrogs were extracted with a sponge for processing (Boughton et al., 2000). If not previously marked, we excised toes to generate individual toe-clip identifications. Toe excision did not appear to hinder treefrog movements nor affect the recapture frequencies of individuals (see Johnson et al., 2007). After processing, treefrogs were returned to the pipes. Several nocturnal monitoring visits revealed that treefrogs captured during the day were absent from artificial refugia at night but frequently returned during the next monitoring interval. Capture data were grouped into four categories based on activity and season for comparisons: (1) during the 'Overwintering' season we recorded no captures (typically November–March); (2) 'Pre-breeding' season captures occurred before the formation of nightly breeding choruses (typ-

ically April–May); (3) 'Breeding' season captures occurred during the period in which breeding choruses occur nightly (typically June–July); and (4) 'Post-breeding' season captures occurred following the cessation of nightly breeding activities (typically July–October).

A subset of large (>8.5 g) individuals ($n = 22$) captured within artificial refugia during the post-breeding season were implanted with 0.85-g radiotransmitters (Holohil Industries Inc.) and relocated on a daily basis to increase the resolution of data regarding habitat use. Although individuals were selected for the study based solely on size (i.e., transmitter was not to exceed 10% of body mass [Richards et al., 1994]), all refugia distance classes except "1 m" were evenly represented in our radio-tracked population. Individuals were implanted with transmitters within 24 h of capture and released after a recovery period of approximately 24 h (see Johnson, 2006 for surgical details). We tracked 10 individuals in 2003 from 5 October to 19 November and 12 different individuals from 30 September to 9 November in 2004. Daily relocations included a detailed description of microhabitat location (e.g., approximate height of frog in tree, position above or beneath leaf litter, etc.), and plotting of the location in UTM coordinates. Radio-implanted individuals were not regularly monitored during the evening. Daily relocation data were compiled into space-use estimates for each individual using the "Animal Movement" extension in ArcView (Hooge and Eichenlaub, 1997) using least-squares cross-validation to determine smoothing factors (Worton, 1989). Of the 22 individuals implanted with radiotransmitters, 20 were tracked for an average of 24 days (± 0.9 SE; $n = 485$ relocations) and followed to their overwintering microhabitat. Upon release, two individuals remained in elevated natural refugia throughout the duration of the study and were never relocated nor were transmitters recovered. These individuals were omitted from analyses due to low numbers of relocation points. We recorded one apparent predation event in which a radiotransmitter was found in the leaf litter several hundred meters from its previous location. Transmitters were not recovered from three individuals

due to presumed battery failure towards the end of the expected battery life. All three of these individuals were later recaptured in our artificial refugia following the overwintering season. For detailed descriptions regarding the success of these intracoelomic implantation surgeries see Johnson (2006).

As temperatures approached freezing, we considered individuals that had not moved for at least five consecutive relocations or that were located beneath soil and leaf litter to have reached their final overwintering microhabitat. Radiotransmitters were subsequently removed and treefrogs were replaced at the point of capture following a brief recovery period (Johnson, 2006). Treefrogs were not revisited at the presumed overwintering site during the winter, but all individuals surviving the radiotracking period were recaptured the following spring in the same artificial refugia from which they were originally captured prior to the implantation of radiotransmitters.

Abiotic Factors

Weather data for the period of the study were downloaded from the National Oceanic and Atmospheric Association (NOAA) website as reported from the Columbia Regional Airport weather station 7 km north of the field site to determine correlations between ambient weather variables and daily captures of adult gray treefrogs in artificial refugia. The relationship between weather variables and refugia use was assessed using linear regression and Pearson correlation analyses. Weather variables to be used in regression comparisons were selected by significant ($P < 0.05$) correlations with artificial refugia captures or microhabitat use of radiotracked individuals. When weather variables were significantly correlated with each other ($P < 0.05$), only the variable most highly correlated with refugia use was included in regression models. Variables tested include daily air temperature (minimum, maximum, mean), daily relative humidity (minimum, maximum), daily wind-speed (5 s maximum, 2 min maximum, mean) and daily total rainfall (day of capture, day of capture-1 [i.e., cumulative rainfall for the day of capture and the day preceding capture], day of capture-2, day of capture-3, and day of capture-4). Daily captures and rainfall data

were square root transformed to satisfy statistical assumptions (Zar, 1999).

Biotic Factors

We measured the DBH of trees in which artificial refugia were placed, with a DBH tape to the nearest 0.1 cm, and binned trees evenly into three size categories ("small" [10.3–18.5 cm]; "medium" [18.6–24.9 cm]; "large" [25.5–50.4 cm]). Additionally, trees were identified to species and grouped into categories ("ash", "elms", "hickories", "other", "red oaks", "sugar maple", and "white oaks"). We analyzed three-dimensional contingency tables of capture frequencies using log-linear models and goodness-of-fit tests (Fienberg, 1970; Zar, 1999) to reveal relationships between tree species categories and tree DBH categories, with either sex of individuals, snout-vent length (SVL) category of individuals ("short male" [28–43.5 mm], "long male" [44–49 mm], "short female" [34–47.5 mm], and "long female" [48–56 mm]), or season of capture (as described above). Observed treefrog capture frequencies were weighted based on the relative proportion of each tree category containing artificial refugia, and by the duration of the season in which the captures were recorded. We minimized sampling zeros by including only the three most abundant tree species categories ("sugar maple", "red oaks", and "white oaks") in the analyses. Mosaic plots depicting the frequencies of observed cell counts from the contingency tables and highlighting deviations from expected proportions were generated as described in Friendly (1994). Mosaic plots were generated using the "vcd" (Meyer et al., 2005) package in R (R Development Core Team, 2005).

RESULTS

Monitoring and Radiotracking

A total of 109 adult treefrogs (42 female and 67 male) and 27 juveniles were captured 737 times in artificial arboreal refugia. Of the 136 treefrogs captured, 101 (74%) were captured on multiple occasions, and 65 of the 101 (64%) treefrogs captured multiple times were recaptured from only a single location. Sixty of the 65 (92%) individuals that were recaptured

in only a single refugium displayed site fidelity and were recaptured following absences (e.g., for foraging in adjacent trees or overwintering) during some monitoring sessions. We observed 107 movements between pipes by 36 individuals, with the majority (60%) of movements occurring between paired artificial retreats ($\bar{x} = 5.04 \text{ m} \pm 0.42 \text{ SE}$) at each distance within transects.

Movements of radiotracked individuals were similarly short in length, as represented by 95 percent kernel space-use estimates that varied from 5.6 m^2 to 2747.8 m^2 across all individuals ($\bar{x} = 530.2 \text{ m}^2 \pm 186.2 \text{ SE}$). Radiotracked individuals moved an average of 8.3 times ($\pm 0.96 \text{ SE}$) with an average of 6.1 m ($\pm 0.71 \text{ SE}$) per move. The average total linear distance moved within foraging grounds by radiotransmitter-implanted individuals was 52.8 m ($\pm 8.61 \text{ SE}$), and the longest single movement recorded was 80.3 m. Many individuals made multiple short distance movements presumably for foraging while temperatures remained above freezing, and a single longer distance movement to reach overwintering microhabitat.

Eighteen individuals were ultimately tracked to the ground for overwintering. Of the remaining four individuals, we were unable to recover three from trees before radiotransmitter batteries expired, and one individual was extracted from a small opening in the distal end of a horizontal white oak (*Q. alba*) branch approximately 9.2 m off the ground. Most overwintering sites consisted of shallow depressions in the soil beneath several inches of leaf litter within which the treefrogs eventually became covered with a small amount of loose soil, presumably following heavy rains. However, a few individuals ($n = 3$) utilized shallow underground small-mammal burrows during the tracking period.

Abiotic Factors

For all weather data regression analyses, we considered each individual capture (or recapture) as an independent event. As justification for the use of recaptures (as opposed to the first capture of each individual), we considered both biological and statistical aspects of the data. First, nocturnal monitoring of artificial refugia (J. R. Johnson, unpublished data) and stomach content analyses (Mahan

and Johnson, 2007; see Discussion) suggest that treefrogs leave the artificial refugia for nightly foraging activities, indicating that any effects of ambient weather conditions on capture frequencies are based on a "decision" to return to refugia and should be independent from one monitoring interval to the next. Second, the effects of capture frequency on the variation in the response variables (Minimum relative humidity [MRH], previous 3 days cumulative rainfall [3DR], and maximum air temperature [MT]) indicate that frogs that were captured many times responded to environmental conditions in the same manner as frogs captured only once based on the lack of a significant relationship between number of captures per individual and the standard deviation of the response variables. Furthermore, variance component estimates (from ANOVA with individual as a random factor) suggest that individuals contributed low levels of variation in MT (10.1%), MRH (15.8%), and 3DR (4.9%) compared to the overall variation observed for the entire population of data. Confirmation that the low levels of individual-specific variation are significantly less than the overall variance component estimated for each variable is provided by 95% confidence intervals.

Minimum relative humidity, previous 3 d cumulative rainfall, and maximum air temperature were the best predictors of the number of treefrog captures, but no single variable explained greater than 16% of the observed variation in capture data when all capture data were combined. MRH ($F_{1,129} = 12.66$, $P < 0.001$, $r^2 = -0.09$) and 3DR ($F_{1,129} = 9.94$, $P = 0.003$, $r^2 = -0.07$) were negatively correlated with pipe captures, and MT ($F_{1,129} = 24.20$, $P < 0.001$, $r^2 = 0.16$) was positively associated with captures. However, when we partitioned the data into four categories (i.e., overwintering, pre-breeding, breeding, and post-breeding) based on observed activity patterns at our sites, we found that 3DR had a greater negative correlation with captures during the pre-breeding season ($F_{1,11} = 6.18$, $P = 0.030$, $r^2 = -0.36$; Fig. 1A), MRH had a greater negative correlation during the post-breeding season ($F_{1,85} = 25.51$, $P < 0.001$, $r^2 = -0.23$; Fig. 1B), and MT had a greater positive association with

captures during the post-breeding season ($F_{1,85} = 29.32$, $P < 0.001$, $r^2 = 0.26$; Fig. 1C). We recorded no captures during the overwintering season (although artificial refugia were not as frequently monitored), and we found no significant relationships between the weather variables and captures during the breeding season.

Radiotracking data suggest that cool days with low MRH resulted in higher proportions of radiotelemetered individuals within leaf litter, and hotter, dryer days were associated with increased use of arboreal retreats (Table 1). High 3DR values during the post-breeding seasons were most highly correlated with increased use of the tree canopy microhabitat by radiotracked individuals, while calendar date was associated with soil microhabitat use (Table 1). However, each weather variable explained relatively small proportions of the variation in treefrog captures and microhabitat use even though statistically significant relationships were found.

Biotic Factors

Twenty-five artificial refugia failed to record a treefrog capture. When considering the use of particular artificial retreats by individual treefrogs, we used unique captures in our analyses because the presence of an individual in a particular refuge is likely not independent from one monitoring period to the next. Tree species, DBH, and sex of capture are not mutually independent ($G = 46.95$, $df = 12$, $P < 0.001$; Fig. 2A), and models of partial independence indicate that species of tree is associated with sex of capture ($G = 22.59$, $df = 10$, $P = 0.012$), while DBH is not ($G = 11.00$, $df = 10$, $P = 0.357$). Standardized Pearson residuals indicated that males were overrepresented in red oak tree captures and females are more abundant than expected in white oak trees.

Tree species, DBH, and size of capture appear mutually independent ($G = 10.51$, $df = 12$, $P = 0.571$). However, when male and female captures were analyzed separately, we found evidence to support an interaction between the size of females and capture-tree characteristics ($G = 22.414$, $df = 12$, $P = 0.033$; Fig. 2B). Models of partial independence indicated that species of tree was not associated with the size of female captures ($G = 13.053$, df

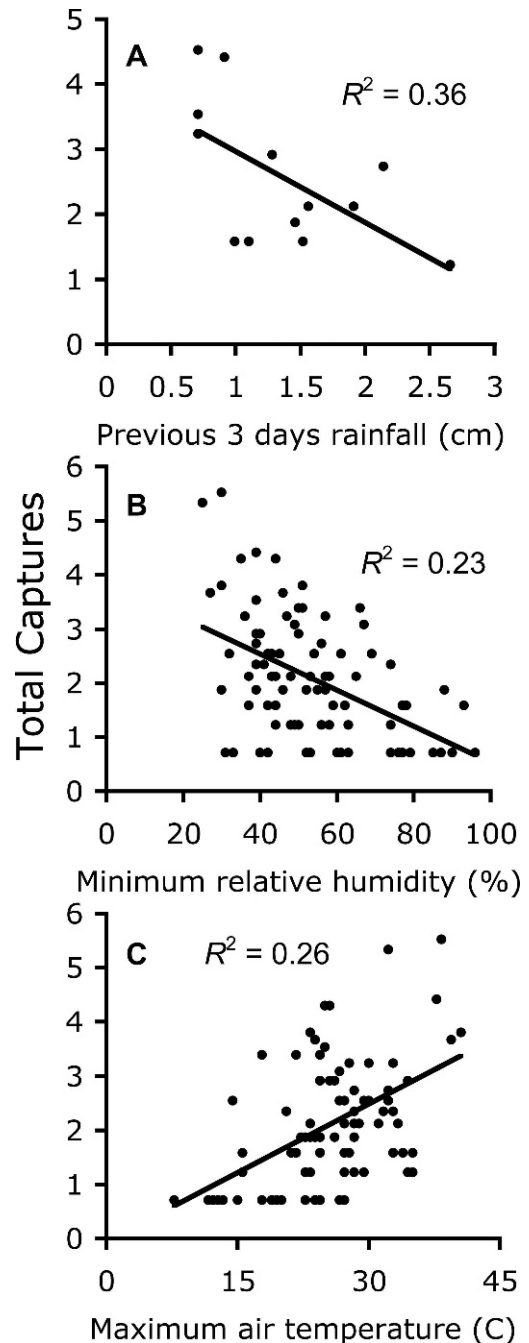


FIG. 1.—Scatter plots of three weather variables most highly correlated with treefrog captures (square-root transformed). (A) Previous cumulative 3-day rainfall during the pre-breeding season, (B) minimum relative humidity during the postbreeding season, and (C) maximum air temperature during the post-breeding season.

TABLE 1.—Correlation (Pearson's R) of weather variables with proportion of individual treefrogs in each microhabitat type during radiotracking.

	Leaves	Soil	Canopy	Artificial retreat
Maximum Daily Temperature	-0.313**	-0.053	0.191	0.337**
Minimum Relative Humidity	-0.102	0.271*	0.054	-0.194
3 Day Rain Accumulation	-0.325**	0.084	0.386**	0.034
Date	-0.211	0.393**	0.094	-0.206

* $P < 0.01$ ** $P < 0.001$

= 10, $P = 0.221$), but that DBH was related to variation in the size of female captures ($G = 18.263$, $df = 10$, $P = 0.051$). Standardized Pearson residuals indicated that small females were more abundant than expected in small DBH trees. The size of male captures was less associated with tree characteristics ($G = 17.444$, $df = 12$, $P = 0.134$).

Tree species, DBH, and season of capture are mutually independent ($G = 7.971$, $df = 12$, $P = 0.787$). Furthermore, seasonality does not appear to affect the association of male captures ($G = 15.373$, $df = 12$, $P = 0.222$) or female captures ($G = 13.278$, $df = 12$, $P = 0.349$) with habitat-tree attributes. Whether a tree was living or dead had no effect on capture frequencies ($G = 0.477$, $df = 1$, $P = 0.490$).

DISCUSSION

It is increasingly clear that the terrestrial habitat adjacent to amphibian breeding sites is important for nonbreeding activities such as migration, dispersal, foraging, and overwintering (e.g., Semlitsch, 1998, 2000, 2008). Thus, population dynamics cannot solely depend on the quality of aquatic habitat, and habitat patches should not be characterized by aquatic habitat alone (Marsh and Trenham, 2001). Further, recent models of population viability indicate that post-metamorphic vital rates and habitat size strongly influence the population dynamics of amphibians (Biek et al., 2002; Halpern et al., 2005; Harper and Semlitsch, 2007). Long-term coexistence of natural wildlife populations and land-use strategies depends on understanding and minimizing the effects of habitat alteration through an improved understanding of important habitat features and behavioral responses to environmental conditions.

Our study identified features of the terrestrial habitat that are utilized by gray treefrogs

and revealed some environmental variables that may influence microhabitat choices of individuals. We calculated relatively small space-use estimates based on radiotelemetry data and recorded frequent movements only between refugia separated by short distances. Few estimates of space-use overlapped among the two years of radiotracking, and none overlapped within years. In general, daily activity appeared to be limited to several adjacent trees, and overwintering sites were in close proximity to arboreal activity locations, indicating that nonbreeding terrestrial habitat for gray treefrogs serves the dual purposes of providing appropriate overwintering and foraging microhabitat. This is perhaps not surprising given that our estimates were based upon post-breeding foraging activities occurring after longer-distance emigration movements had ceased. Our results would likely be quite different if animals were implanted with radiotransmitters while still at breeding sites, as gray treefrog migration distances of several hundred meters have been reported (Johnson et al., 2007) and dispersal movements greater than 1 km have been recorded in congeners (e.g., *H. arborea*: Carlson and Edenham, 2000).

For most amphibians, microhabitat choice is a very important decision due to physiological constraints (Tracy et al., 1993). For ectotherms, ambient temperature plays a critical role in determining activity levels (Wygoda, 1989), as does evaporative water loss (Wygoda, 1984) given the permeability of amphibian skin. Not surprisingly, environmental variables that affect an individual's water balance were most correlated with the use of different categories of microhabitat by animals implanted with radiotransmitters and capture frequency in artificial refugia. Early in the fall, when conditions were moist (i.e., high MRH and 3DR) radiotelemetered individuals

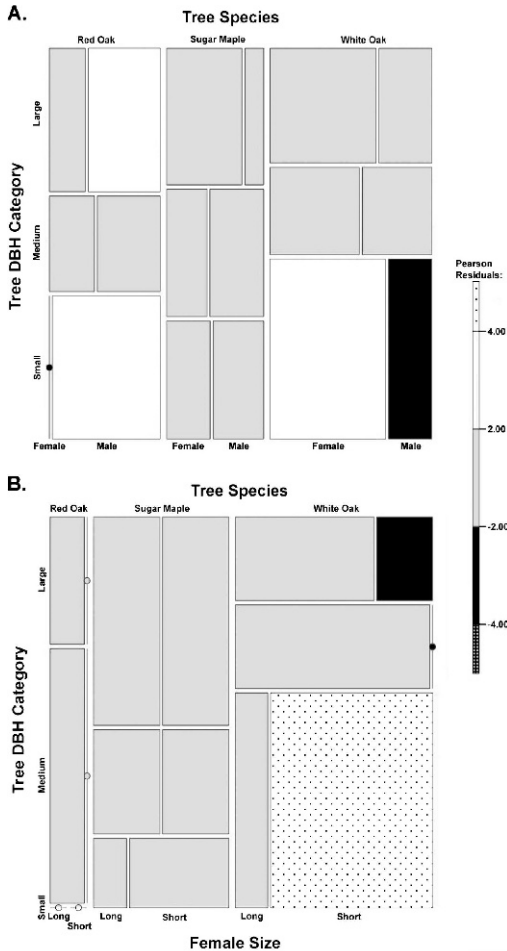


FIG. 2.—Mosaic plots depicting the observed frequencies of captures as distributed among tree species category, tree DBH category, and either (A) sex of capture, or (B) SVL category of female capture. The size of tile is proportional to the frequencies of captures. Observed capture values with Pearson's residuals less than -2 indicating significantly fewer captures than expected are depicted as black-filled tiles. Observed capture values with Pearson's residuals greater than $+2$ indicating significantly greater captures than expected are depicted as white-filled tiles. Pearson residuals greater than $+4$ are depicted as white tiles with black stippling.

tended to move upwards in the canopy, and captures in artificial refugia decreased, which supports the suggestion that treetops may be the preferred microhabitat for foraging, escaping predation, or both (Ritke and Babb, 1991). It remains unknown whether treefrogs are able to forego the need for diurnal refuge in tree cavities during moist days, or whether

they used natural refugia when absent from our artificial refugia. In any case, hotter and drier conditions (i.e., high MT or low MRH) resulted in a trend towards increased use of artificial retreats (and presumably natural tree cavities as well) during the pre- and post-breeding seasons, perhaps to take advantage of the humid conditions within, and decrease evaporative water loss, as suggested by Buchanan (1988) and Stewart and Rand (1991). Furthermore, we found that individuals enclosed within artificial refugia maintained hydration, while those exposed to ambient conditions in screened refugia quickly desiccated (<6 h: R.D. Mahan and J.R. Johnson, unpublished data).

The choice to use arboreal refugia may not be driven simply by the need to maintain proper water balance, as Mahan and Johnson (2007) found that all individuals captured in artificial refugia contained full stomachs. Because treefrog stomach contents are rapidly digested (Kilby, 1945), the presence of stomach contents indicates that retreats were utilized between nocturnal foraging bouts. Based on the types of insects collected from the stomach samples analyzed by Mahan and Johnson (2007), it appears that foraging occurs arboreally, and thus the use of diurnal retreats could be twofold: to provide escape from hot/dry weather and to provide access to foraging sites.

Artificial refugia captures decreased as the weather cooled, and radiotelemetered treefrogs moved towards the ground in apparent preparation for movement to hibernation sites in the soil beneath the leaf litter. Gray treefrog overwintering locations have previously been only anecdotally reported (Burkholder, 1998), but our data demonstrate that the ground is the preferred microhabitat for overwintering of gray treefrogs. The soil beneath leaf litter likely provides a moister and/or more thermally appropriate location than tree cavities to face cold winter temperatures even though gray treefrogs display some degree of freeze tolerance (Layne, 1999). Spending the winter months on the ground likely means that leaf litter depth is positively associated with the quality of particular overwintering locations and that habitat alteration could affect the distribution and abundance of appropriate overwintering

microhabitat (Zheng et al., 2000). Based on our results, we believe that alteration of habitat that results in changes to the microclimate experienced by individuals (e.g., reduced canopy cover, treehole abundance, leaf litter depth) could affect the ability of treefrogs to forage arboreally and overwinter on the forest floor.

Treefrogs do not appear to choose their habitat-trees randomly, as we found differences in capture frequencies among tree species and DBH categories. Based on relative proportions and expected values generated from the contingency table analyses, we found a greater than expected number of male captures in artificial refugia placed on red oak trees and more females than expected in white oak tree refugia. The reasons for this relationship are unknown, but the number of different individuals encountered during diurnal use of artificial refugia is likely related to the proximity of high quality foraging sites, and/or the abundance of nearby natural tree cavities. The relative contribution of each sex or size category to the total number of different individuals captured in a refugium is likely the result of trade-offs associated with the motivation to pursue high quality resources and the ability to compete for the acquisition of such resources. The dynamics of competition between male treefrogs for calling perches has been well established (e.g., Fellers, 1979); however, the behaviors associated with defense of retreats not associated with reproduction are less understood. Buchanan (1988) observed resident male *H. squirella* reacting aggressively towards diurnal retreat intruders, and Stewart and Rand (1991) attributed observed territorial behavior by both male and female *Elutherodactylus coqui* at retreat sites to the need for protection from desiccation and predation, and to ensure spacing for adequate foraging resources.

If we assume that larger individuals are more successful at obtaining high quality retreat sites (as is suggested by Buchanan [1988] and Stewart and Rand [1991]), females should have the ability to acquire refugia in higher quality locations than males due to sexual size dimorphism. Interestingly, previous work has demonstrated that gray treefrogs captured in white oak trees produced larger stomach content

masses than in red oaks or sugar maples (Mahan and Johnson, 2007), indicating that white oak trees may provide improved foraging resources relative to other species of tree. However, we have yet to empirically determine the relationship between insect abundance and tree species at these sites. We also found that small females were less represented in medium and large DBH trees than expected by chance. If smaller females are poorer competitors, we can hypothesize that larger diameter trees support better foraging habitat than smaller diameter trees. Large diameter trees are most likely older, and contain a larger number of natural tree cavities (Fan et al., 2003a, 2005) that also enhance microclimate characteristics. But Mahan and Johnson (2007) concluded that DBH was not related to stomach content mass of frogs captured in artificial refugia.

Unfortunately, we have no clear prediction regarding expected patterns of refugia use based on tree diameter. For example, we may expect fewer captures in our artificial refugia placed in large trees simply because suitable alternative to artificial refugia already exist in those trees. Such a relationship is supported by our observed deficit of individuals captured from dead trees (i.e., snags) versus live trees, as dead trees almost certainly contain a greater number of cavities (Fan et al., 2003b). Perhaps we should expect more captures as tree diameter increases because more treefrogs would identify large trees as appropriate sites in which to search for natural refugia or because increased surface area provides habitat for a greater number of individuals. The preferential use of particular species or sizes of trees could have important implications concerning forest management strategies if the selective removal of trees coincides with the loss of high quality (or frequently used) treefrog microhabitat.

Our data indicate that gray treefrogs utilize terrestrial habitat adjacent to breeding sites for a variety of activities including foraging and overwintering. Trees are a critical component of the terrestrial habitat of gray treefrogs through the provisioning of canopy locations in which to forage, cavities in which to prevent diurnal desiccation, and leaf litter to protect overwintering treefrogs from cold and dry winter conditions. Future, manipula-

tive investigations of factors affecting arboreal anuran abundances and survival (tree species composition, tree cavity abundance, leaf litter depth, etc.) will improve our ability to define habitat requirements and make informed land management decisions for arboreal amphibians in deciduous forests.

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