MULTI-SCALE INVESTIGATIONS OF GRAY TREEFROG MOVEMENTS: PATTERNS OF MIGRATION, DISPERSAL, AND GENE FLOW

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by
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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

MULTI-SCALED INVESTIGATIONS OF GRAY TREEFROG MOVEMENTS:
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a candidate for the degree of Doctor of Philosophy

and hereby certify that in their opinion it is worthy of acceptance.

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ABSTRACT

The movement of individuals within and among populations has long been recognized as an important determinant of the local and regional persistence of species. The field of metapopulation biology is primarily concerned with understanding the dynamics of systems of populations that exchange individuals along a continuum of inter-population movement probabilities. Metapopulations consist of multiple populations within discrete habitat ‘patches’ separated by inhospitable ‘matrix’ habitat. Movements within patches are migratory and movements among patches (i.e., through matrix) represent dispersal and ultimately gene flow. Theoretical models of metapopulation dynamics require information regarding habitat patch size/quality, and matrix composition to estimate extinction and recolonization probabilities at local and regional scales. However, basic information regarding movement capabilities and habitat use are generally lacking for many taxa.

Fragmentation of terrestrial landscapes has exacerbated the need to understand the effects of changes to the core habitat of mobile organisms and has led to an increase in the use of metapopulation theory in conservation biology. Pond-breeding amphibians represent an appropriate group in which to study metapopulations because the local habitat patch is centered on a breeding pond, but adults spend much of their lives in adjacent terrestrial habitat. Further, amphibians are experiencing worldwide population declines resulting from many factors, including habitat loss. From an ecological and conservation perspective, an understanding of the effects of variation in patch characteristics, inter-pond distance and matrix composition are crucial to implementing programs aimed at protecting natural amphibian population dynamics and preserving biodiversity.

I have used direct (e.g., mark-recapture, radiotelemetry) and indirect (e.g., F_{st} estimates) methods to determine the capacity for movements by adult and juvenile gray treefrogs (*Hyla versicolor*). Specifically, I have determined the spatial scale of migratory movements, the location of overwintering locations, and the biotic and abiotic factors determining microhabitat use. Further, I have demonstrated the effects of matrix composition on the frequency of inter-pond movements and elucidated historical patterns of gene flow across central Missouri.

My data indicate that migration through terrestrial habitat adjacent to breeding sites is extensive and significantly different for males and females. Furthermore, dispersal success is affected by matrix composition and inter-pond distance, but juvenile and adult movements are differently inhibited. Lastly, the type of metapopulation dynamics exhibited by associations of gray treefrog populations depends upon the degree of geographic isolation and the presence of barriers to movement.

Chapter 1

DEFINING CORE HABITAT OF LOCAL POPULATIONS OF THE GRAY TREEFROG (*HYLA VERSICOLOR*) BASED ON CHOICE OF OVIPOSITION SITE

Jarrett R. Johnson and Raymond D. Semlitsch

ABSTRACT

Concern over amphibian population declines and loss of terrestrial and aquatic habitat have emphasized the need to define habitat requirements for each stage in a species' life history. The realization that pond-breeding amphibians spend most of their lives in the terrestrial environment suggests the need to protect terrestrial as well as aquatic habitat. Many studies on amphibian populations have focused on emigration from breeding sites to define habitat use, however these studies do not typically elucidate terrestrial activities of adults within the breeding season. We measured colonization rates of artificial pools by gray treefrogs (*Hyla versicolor*) at multiple distances from natural breeding ponds. We found a non-random distribution of egg deposition among distances, with 95% of eggs deposited within 15 m of the breeding pond. Additionally, we found that the time to first colonization of artificial pools increased with respect to distance. Our results indicate that adult gray treefrogs may travel up to 200 m within a breeding season, and that multiple breeding ponds may be considered part of a single population. We suggest

that a minimum core terrestrial habitat of 60 m surrounding breeding sites is appropriate for protection of local populations of gray treefrogs.

INTRODUCTION

Concern over the loss and fragmentation of natural habitat has led to increased interest in metapopulation dynamics in the field of conservation biology (e.g., Hanski and Gilpin 1991; Pulliam 1988). Conservation-oriented metapopulation studies of amphibians are of particular importance due to widespread concern over population declines and local extinctions (e.g., Houlahan et al. 2000). Additionally, populations of pond-breeding amphibians are naturally spatially clumped due to their dependence on aquatic habitat for breeding and larval development, and are well-suited for investigation of metapopulation dynamics (e.g., Gill 1978). The preservation of metapopulation dynamics is a critical aspect of conservation plans because recolonization of extinct patches is important for long-term preservation of metapopulations (e.g., Semlitsch 2000, 2002). The probability of extinction increases as the probability of recolonization of unoccupied habitat patches decreases with loss and fragmentation of natural habitat.

The historical view of amphibian populations considered each breeding site as a discrete population (Gill 1978; Sjögren 1991; Sjögren-Gulve 1994). This view of amphibian populations facilitated application of simple metapopulation models similar to those envisioned by Levins (1970), in which each subpopulation consisted of a discrete aquatic breeding habitat patch and movement between any two breeding sites was interpreted as a dispersal event. This ‘ponds as patches’ view has traditionally been accepted because the aquatic habitat of pond-breeding amphibians can easily be characterized and defined (Marsh and Trenham 2001), and because amphibians are typically assumed to exhibit natal pond philopatry and limited dispersal ability (e.g., Berven and Grudzien 1990). However, direct monitoring of individual movements and indirect estimates of movements from molecular markers suggest that individuals of some amphibian species can move between two or more adjacent breeding sites (Breden 1987; Sinsch 1992, 1997; Trenham et al 2001). Thus, the 'ponds as patches' model may not adequately characterize all amphibian populations. The development of realistic metapopulation models requires information on spatial dimensions of subpopulations as defined by movements in the terrestrial environment adjacent to breeding sites. Determination of the local patch size is an important step in delineating the critical terrestrial habitat area necessary for long-term survival of populations through maintaining dispersal and recolonization processes.

In our study, we identified the area around breeding ponds used by adult gray treefrogs (*Hyla versicolor*) during a single breeding season by monitoring colonization of artificial ponds at multiple distances from natural breeding sites. The terrestrial environment may be especially important to the gray treefrog because it breeds over a period of several months (Johnson 2000; Ritke et al. 1990). During the breeding season, gray treefrogs use the terrestrial habitat for shelter and foraging between breeding bouts, as well as for arboreal calling sites surrounding the breeding pond. Previous studies have reported that movements of *Hyla gratiosa* (Murphy 1994) and *H. chrysoscelis* (Ritke et al. 1991) between breeding sites within a breeding season are rare. However, most previous studies providing data on within-breeding season movements of hylids (Harris

1975; Freda and Gonzalez 1986; Jameson 1957; Perrill 1984) have not been aimed at providing a relevant end-point to individual movements in the context of population biology, or in identifying the importance of the terrestrial environment for amphibians. Our study provides direct evidence of within breeding season movements through the terrestrial environment that result in reproduction. Identification of oviposition events outside the natural breeding site will help to quantify the spatial dimensions of gray treefrog subpopulations and aid in determination of appropriate protection guidelines for core habitat adjacent to amphibian breeding sites.

METHODS

Study species

The gray treefrog (*Hyla versicolor*) breeds in ephemeral or fishless ponds from early April to early July in Missouri (Johnson 2000). Gray treefrogs have been used in other studies using artificial ponds because females readily deposit eggs in small pools (Ritke et al. 1990; Ritke 1991; Ritke and Semlitsch 1991; Resetarits and Wilbur 1989). Males have been observed to call from the edge of artificial ponds and attract females to the pool (Resetarits and Wilbur 1991). Females deposit multiple batches of 20-90 eggs until the full compliment has been laid (Ritke et al. 1990), and eggs float on the surface of the water for approximately 24 hours, or until they are disturbed. Eggs hatch in 4-5 days (Johnson 2000) depending on the temperature, and larvae are free-swimming shortly after.

Other amphibians that occur at our study sites are the spotted salamander

(*Ambystoma maculatum*), smallmouth salamander (*A. texanum*), southern leopard frog (*Rana sphenocephala*), green frog (*R. clamitans*), bullfrog (*R. catesbeiana*), chorus frog (*Pseudacris triseriata*), spring peeper (*P. crucifer*), cricket frog (*Acris crepitans*), and American toad (*Bufo americanus*). Most of these species do not breed at the same time as gray treefrogs and the eggs of those that do are easily distinguished from gray treefrog eggs. Only three juvenile individuals of one other species (green frog) were found in the treatment pools during the course of our study.

Study Design

We placed two experimental transects composed of seven (1.52 m diameter, 30 cm deep) plastic wading pools in the terrestrial habitat surrounding each of three natural breeding ponds located at the Baskett Wildlife Research Area in Boone County, Missouri. Ponds A and B are within 60 m of each other, and pond C is separated from ponds A and B by one kilometer. All three natural breeding ponds are at least 500 m away from other breeding sites and all wading pools were at least 300 m away from breeding sites not included in the study. All three breeding ponds are fishless and have variable hydroperiods, and pond B dried and filled several times throughout the study. We do not believe that pond drying influenced our results, as Ritke (1991) reported that drying of breeding sites did not stimulate dispersal of *Hyla chrysoscelis*. The terrestrial habitat surrounding all three ponds consists of secondary growth oak (*Quercus spp.*)/hickory (*Carya spp.*) canopy with maple (*Acer spp.*)/cedar (*Juniperus spp.*) understory. Plastic wading pools were placed at 1, 5, 15, 35, 60, 100, and 200 m from the breeding ponds (Figure 1). Wading pools were arranged in a staggered fashion to maximize the distance between experimental pools both within and between transects and limit movement from pool to pool (Table 1). Distances between wading pools were maximized both within and between adjacent transects, while maintaining the appropriate distance from the breeding pond. All pools were placed at a level location to avoid confounding slope and distance. Each pool was also placed at the base of a large diameter (mean=30.9 cm) oak, maple, or hickory tree to provide elevated refuges and foraging sites for adult treefrogs.

Wading pools were placed at the study sites on 23 May 2001 after the breeding season had begun, and adult gray treefrogs were seen and heard at all three breeding ponds. All wading pools were filled by garden hose from a tank truck using tap water and from rainwater to approximately 20 c m deep. After all pools in a transect were filled, wading pools were checked daily until the first oviposition event occurred. Subsequently, wading pools were monitored every three days and all eggs found in each pool were counted. Eggs were counted individually in small containers, after which they were returned to the corresponding natural breeding pond. Occasionally, not all eggs were detected and newly hatched tadpoles were found upon subsequent monitoring. Tadpoles were assigned to that pool's egg count from the previous monitoring-day if newly hatched with yolk sac or to the second previous monitoring-day if larger and without yolk sac. Eggs, rather than tadpoles, were counted every third day to avoid the biases of hatching mortality and insect predation on tadpoles. No attempt was made to remove insect predators from the pools, but vegetative debris that had fallen into the pools was removed each time they were checked. We assumed that females depositing eggs came from the closest natural breeding pond and the adjacent forest. Monitoring of wading pools ceased on 9 August 2001 after a period of 18 days with only two oviposition events.

We calculated two estimates of the number of females ovipositing eggs based on average female clutch size from western Tennessee (2,060 eggs; Ritke et al. 1990) and central Missouri (1,018 eggs; S. James unpublished data), because we did not attempt to directly observe females depositing eggs in the pools. We performed Kruskal-Wallis tests with a correction for tied ranks to determine if the number of eggs deposited, number of females ovipositing, and number of oviposition events differ among treatment distances. We also performed survival data analyses in which survival time is defined as the time to the occurrence of an event. In our study, the 'event' was the time to first colonization of wading pools. These 'time-to-colonization' curves were compared to determine if the number of days to first colonization varied among treatment distances.

RESULTS

A total of 52,102 eggs and tadpoles were counted in pools from 29 May through 9 August 2001. There were 58 separate oviposition events on 55 different days. The first oviposition event occurred in a 5 m pool after seven days. Kruskal-Wallis tests for each transect show that the average number of eggs deposited (H_c=15.68, df=5, P<0.01), number of females ovipositing (H_c=15.68, df=5, P<0.01), and number of oviposition events (H_c=15.51, df=5, P<0.01) differed across treatment distances. Figures 2, 3, and 4 indicate that the average number of eggs deposited, estimated average number of females ovipositing, and average number of nights with oviposition events at each site decrease with distance from the breeding ponds. Analysis of 'time-to-colonization' data using a

Generalized Wilcoxon Test (Lee 1992) revealed that the time to first colonization differed among distances (X^2=25.05, *df*=6, *P*<0.001). Figure 5 shows that the average number of days to first oviposition increases with distance from natural ponds. Pairwise comparisons of individual 'time-to-colonization' curves based on the Kruskal-Wallis Test (Lee 1992) showed that the time to colonization increased as distance from the natural breeding site increased, but not in all instances (Table 2). More than 95% of all eggs were deposited within 15 m of the breeding ponds. No eggs were ever found in the pools at 100 m from the natural breeding ponds and eggs were found in a 60 m pond only during the final days of the study. Eggs were recorded on two occasions in a single 200 m treatment pool. We have included results from the 200 m pool in our analyses, however we question whether the individuals that colonized that pool originated from the breeding ponds under investigation due to the timing of colonization and their proximity to a small stream.

DISCUSSION

Gray treefrogs breed over a period of several months during the summer in Missouri. The terrestrial habitat adjacent to breeding sites provides food and shelter throughout the prolonged breeding season. Other studies have shown that treefrogs move to and from a single breeding pond within a breeding season (e.g., Harris 1975; Murphy 1994). Amphibian movements around the breeding habitat can be part of normal foraging behavior within an individual's home range (Gibbons and Bennett 1974), or repeated movements to the pond to deposit additional clutches of eggs (Perrill and Daniels 1983;

Wells 1976). Female gray treefrogs are only present at the breeding pond on nights in which they mate but may return multiple nights during a breeding season (Godwin & Roble 1983; Sullivan & Hinshaw 1992). Male gray treefrogs spend a larger proportion of time at the breeding pond, but all males are not found at the breeding pond on every night of the breeding season that males are calling (Fellers 1979; Ritke & Semlitsch 1991).

It is unclear where adult gray treefrogs go when not attending the chorus at breeding ponds. In our study, oviposition was observed at all treatment distances up to 200 m into the terrestrial habitat, but most breeding activity was within 15 m from the natural breeding pond. Whether treefrogs purposefully moved to the artificial pools from the natural breeding pond, or encountered the pools during other activities away from the breeding pond remains undetermined. Males that moved away from the natural breeding pond to forage may have encountered a wading pool and attracted females that were returning to the natural breeding pond to deposit additional clutches. Alternatively, amplectant pairs may have encountered the artificial pools after leaving the male's perch site at the natural breeding pond. Sullivan and Hinshaw (1992) found that amplectant pairs of gray treefrogs moved along the margin of the pond before oviposition. Regardless of the mechanism of colonization, each female that visited the artificial pools moved some distance through the terrestrial habitat surrounding the natural ponds.

For the 2001 breeding season, we calculated 58 separate gray treefrog oviposition events at our artificial pools, and counted 52,102 eggs and tadpoles. The extent of artificial pool use in this study indicates that gray treefrogs do not exhibit strict breeding pond fidelity and readily breed in novel locations within a breeding season. Thus, the 'ponds as patches' metapopulation model is not strictly applicable, and gray treefrog subpopulations can consist of clusters of nearby ponds that exchange individuals within each breeding season. Sinsch (1992) conducted a *Bufo calamita* metapopulation study in which adjacent breeding sites were grouped into distinct breeding areas. Sinsch (1992) determined that most adult males stayed within a single breeding area throughout the breeding season, but also recorded exchange of individuals between breeding areas over the period of several breeding seasons. Our data suggest that short distance movements between breeding sites by adults may be more common than generally perceived, and investigations of amphibian metapopulations should not assume *a priori* that each pond is a discrete breeding unit during a breeding season.

We found that the number of days until first colonization of artificial pools was positively related to distance from the natural breeding site. Thus, the probability of colonization of new ponds within a breeding season decreases as distance from the main breeding pond increases. This result suggests that the amount of terrestrial habitat used within a breeding season is confined to a quantifiable area surrounding breeding sites. Freda & Gonzalez (1986) determined that daily movements of *Hyla andersoni* were contained within 20 m of breeding ponds, and that most individuals remained within 70 m of the breeding pond throughout the breeding season. Kramer (1973) recorded movements up to 58 m into the terrestrial habitat surrounding breeding ponds, and movements up to 195 m between adjacent breeding sites for *Pseudacris triseriata* within a breeding season. Other studies of treefrog movements have also found that terrestrial movements during the breeding season occur at a relatively small scale (Jameson 1957; Ritke et al. 1991). Our findings correspond with previous studies and indicate that patches of aquatic habitat and adjacent forest can explicitly define local populations of the gray treefrog. The results of our study show that efforts to protect and maintain populations of amphibians should not be limited to the aquatic breeding habitat, and that use of terrestrial habitat surrounding breeding sites by amphibians also could be considered important.

For the gray treefrog in Missouri, we suggest that protection of terrestrial habitat up to a 60 m radius from aquatic breeding habitat will provide the minimum terrestrial habitat necessary to contain the majority of within-breeding season activities. However, we present these data knowing that our approach to monitoring the distribution of adult treefrogs in the terrestrial habitat surrounding breeding ponds may have led to a biased interpretation of actual habitat use. By creating new breeding sites near the existing pond, we may have eliminated the need for longer-distance movements to locate suitable alternative breeding habitat and therefore underestimated the amount of terrestrial habitat needed to encompass a single population. Alternatively, if individuals were capable of detecting new breeding sites at great distances, the wading pools may have drawn individuals farther than usual from the natural breeding site. Thus, leading to an overestimation of the terrestrial habitat typically used by adult treefrogs during the breeding season. Nevertheless, our study has demonstrated that terrestrial habitat surrounding breeding sites is utilized by adult gray treefrogs within a single breeding season and may be important for population persistence.

As with any study regarding habitat use, our results are dependent upon the quality of habitat in which the study was conducted. Our study sites were enclosed in closed canopy, oak/hickory forest that is approximately 100 years old. Mature forest is good-quality habitat for gray treefrogs because it provides refugia during the breeding season while individuals are not foraging or breeding, and overwintering sites during the non-breeding season. The availability of refugia in the terrestrial landscape almost certainly influenced the movement distances of adult treefrogs in our study. In a landscape containing more widely separated refugia, we may have observed reproductive events at greater distances from the natural breeding site, due to a greater frequency of individuals making long-distance movements to reach appropriate terrestrial habitat. While our suggestion of a 60 m radius of core breeding habitat protection is a minimum estimate in continuous forest habitat, it is likely not broadly applicable to degraded environments, but can be used as a starting point for further investigations regarding species or area-specific conservation management.

While our study provides information on the extent of within breeding season adult movements, the extent of terrestrial habitat use between consecutive breeding seasons remains unresolved, and would be better-studied using direct methods for following individuals in the landscape (i.e., radiotelemetry, mark-recapture). Non-breeding season terrestrial habitat use (e.g., overwintering sites) certainly could extend further from the breeding pond than the distances we recorded within a single breeding season, and has been investigated for pond-breeding salamanders (Semlitsch 1998; Trenham 1998). Semlitsch and Bodie (2003) summarize results from the literature regarding terrestrial habitat use by local populations of amphibians, and report a mean minimum (159 m) and maximum (290 m) distance of migration that exceeds our observed movements within a single breeding season. Furthermore, there is evidence that juveniles accomplish the majority of dispersal between breeding sites (Gill 1978; Breden 1987 Berven and Grudzien 1990), and that the distances moved by juveniles between populations may even be greater than those traveled by adults within local populations (Breden 1987). Our data show that suitable terrestrial habitat surrounding breeding sites can lead to colonization of new breeding sites and may help to buffer local populations of treefrogs from extinction. A better understanding of terrestrial habitat requirements at each life history stage is critical for effective amphibian conservation planning.

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Table 1A. Average distance between wading pools within each transect (*N*=6). **B.** Average distance between wading pools between adjacent transects within study sites (*N*=3).

A.

Distance	1 m	5 m	15 m	35 m	60 m	100 m	200 m
1 m	0	13	21	43	66	108	196
5 m	13	0	13	36	57	100	188
15 m	21	13	0	24	48	90	179
35 m	43	36	24	0	30	72	160
60 m	66	57	48	30	0	50	135
100 m	108	100	90	72	50	0	91
200 m	196	188	179	160	135	91	0

B.

Distance	1 m	5 m	15 m	35 m	60 m	100 m	200 m
1 m	13	22	26	46	71	114	205
5 m	19	25	31	49	72	118	207
15 m	29	33	37	48	71	119	206
35 m	49	50	51	51	70	120	202
60 m	72	70	67	59	71	120	196
100 m	113	109	105	90	88	127	184
200 m	197	191	186	168	155	174	188

Table 2. Multiple comparisons of 'time-to-colonization' curves based on the Kruskal-Wallis Test. Colonization events at 60 m, 100 m, and 200 m distances were omitted because of low sample sizes. Significant *P*-values indicate differences in the time elapsed before ponds at treatment distances were colonized.

Comparison	*P*
1 to 5	NS
1 to 15	<0.05
1 to 35	<0.05
5 to 15	<0.05
5 to 35	<0.05
15 to 35	NS

Figure 1. Schematic drawing of a breeding site divided into staggered transects. All three breeding sites were divided into two transects and pools were arranged in a similar fashion. See Table 1 for average distances between wading pools as determined by GPS coordinates. Breeding pond and wading pools are not drawn to scale.

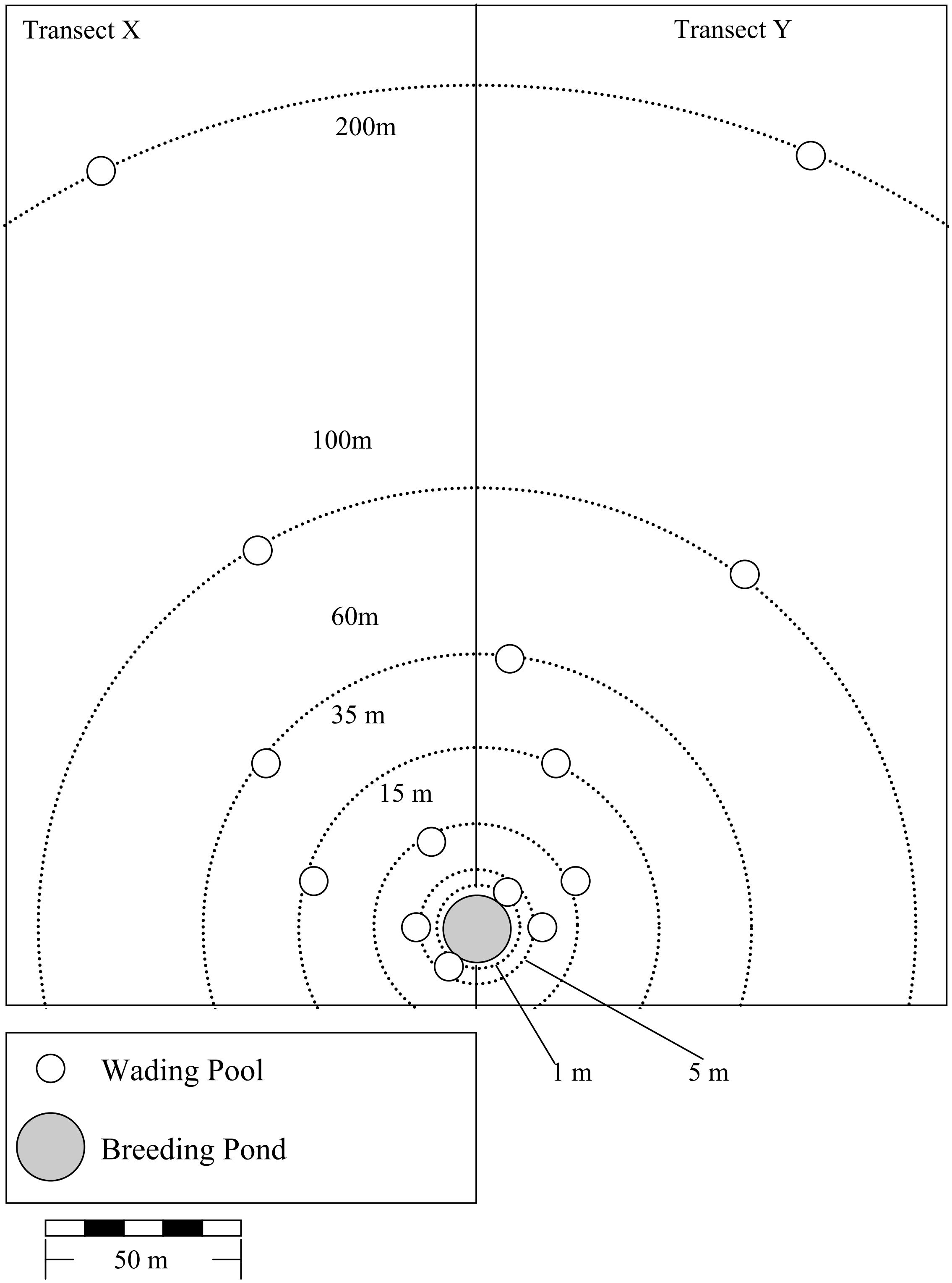

Transect X
Transect Y
200m
100m
60m
35 m
15 m
1 m
5 m
Wading Pool
Breeding Pond
50 m

Figure 2. Average number of *Hyla versicolor* eggs (+SE) counted at each pool in relation to distance from natural breeding pond.

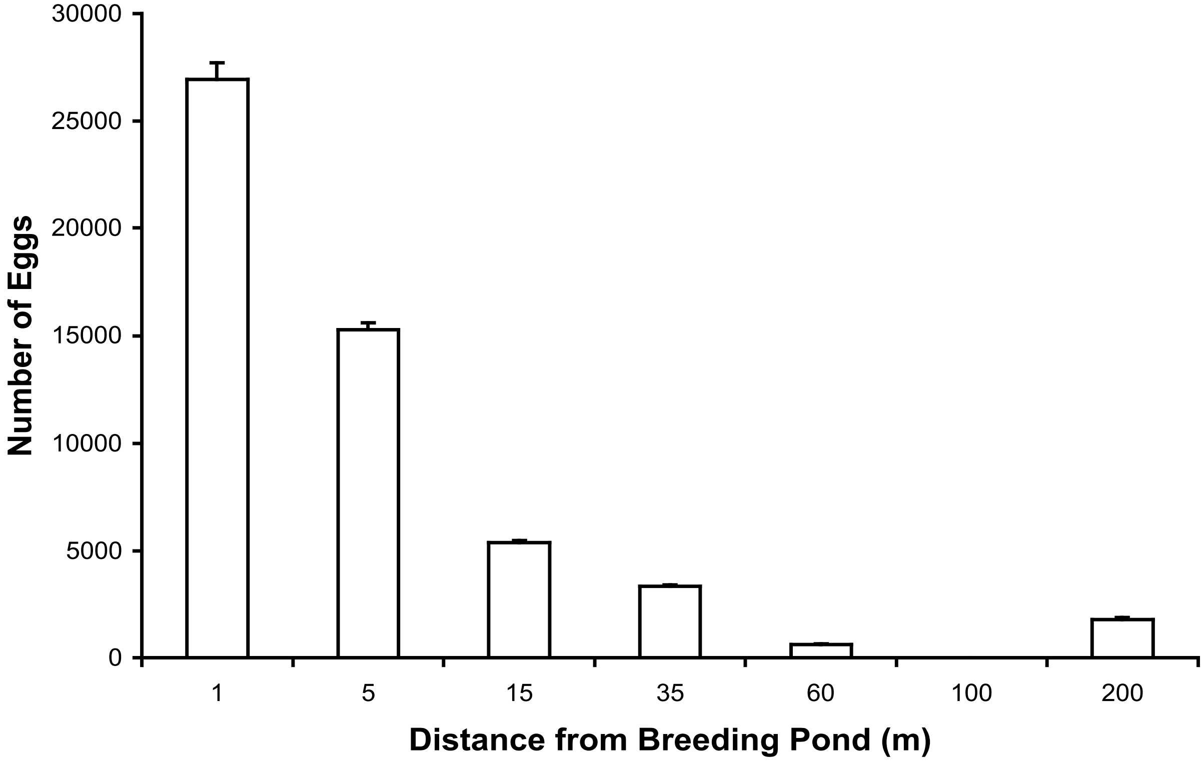
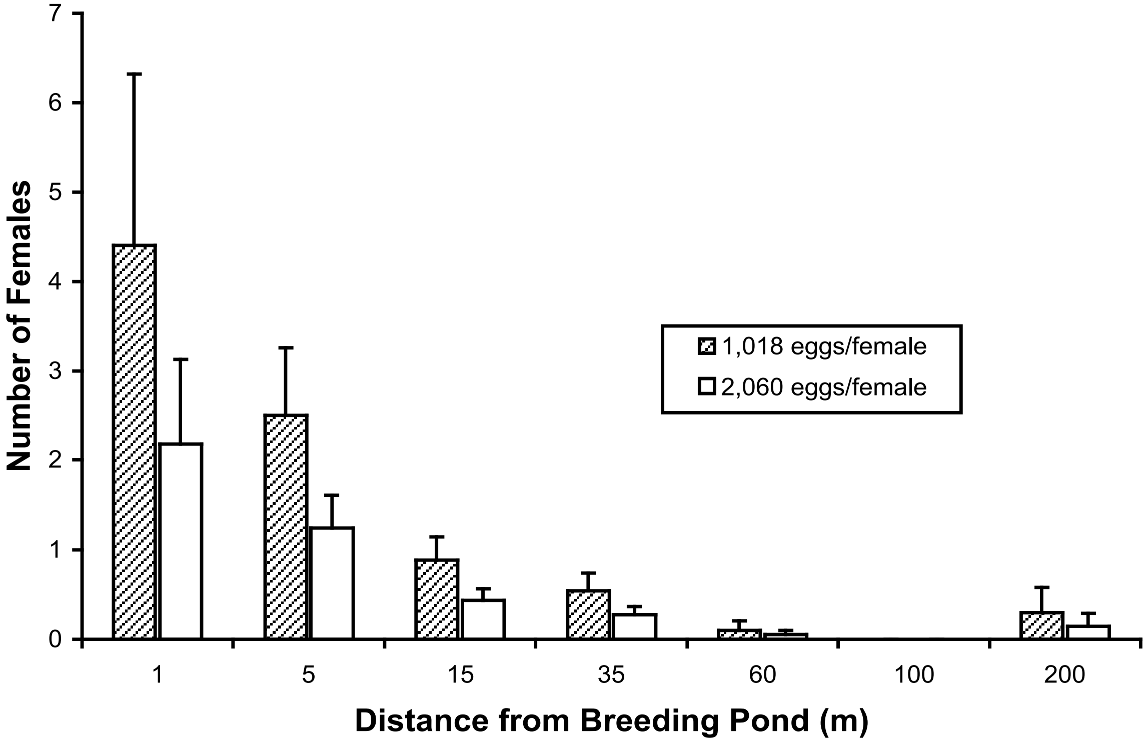

Number of Eggs
30000
25000
20000
15000
10000
5000
0
1
5
15
35
60
100
200
Distance from Breeding Pond (m)

Figure 3. Estimated average number of female *Hyla versicolor* depositing eggs (+SE) in wading pools at each distance from natural breeding ponds based on average female clutch size from western Tennessee (2, 060 eggs) and central Missouri (1, 018 eggs).



7
6
5
4
3
2
1
0
Number of Females
▨ 1,018 eggs/female
☐ 2,060 eggs/female
1
5
15
35
60
100
200
Distance from Breeding Pond (m)

Figure 4. Average number of nights in which an oviposition occurred, at each distance (+SE) from the natural breeding site.

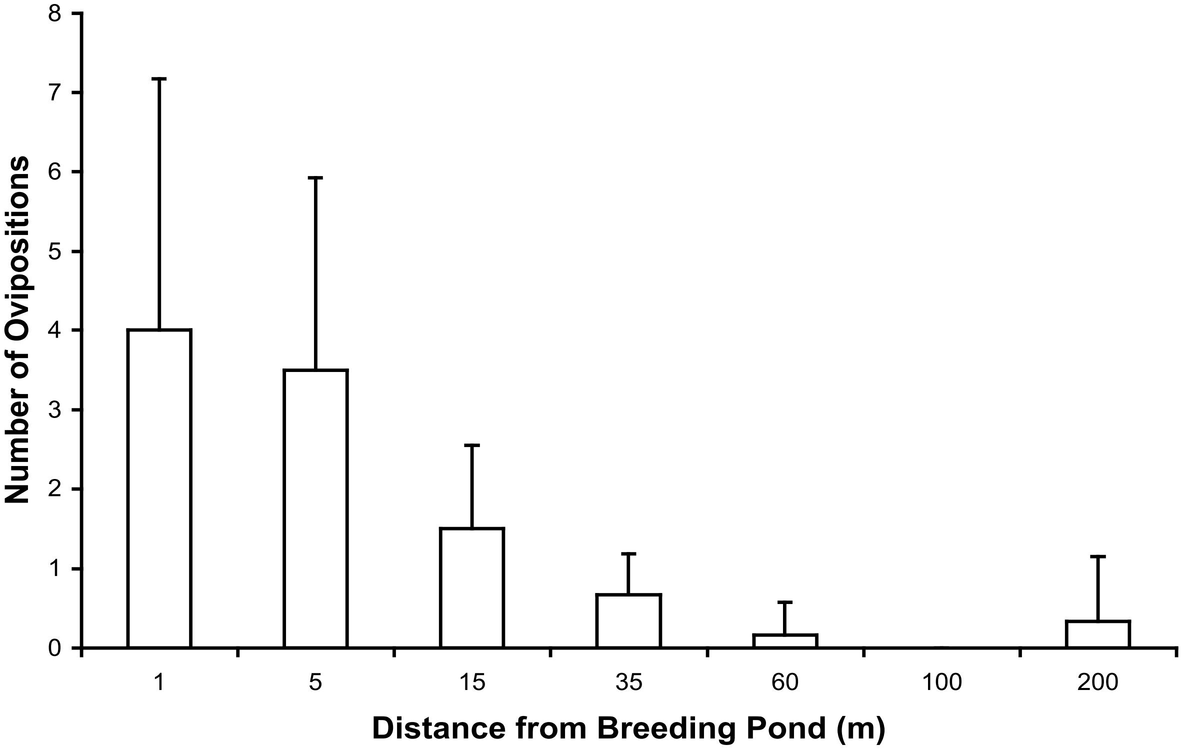
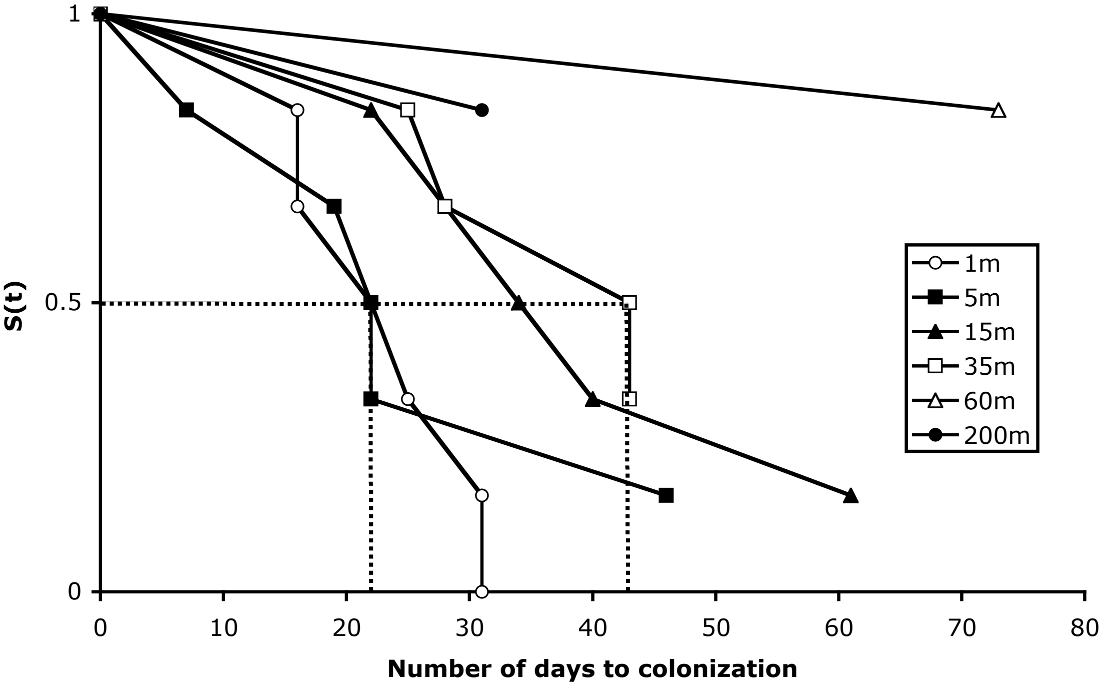

8
7
6
5
4
3
2
1
0
Number of Ovipositions
1
5
15
35
60
100
200
Distance from Breeding Pond (m)

Figure 5. Number of days until first colonization of wading pools by *Hyla versicolor* represented as survival curves. Ponds that were never colonized were censored and included in the analysis. Dotted lines indicate the median time-to-colonization for each of the curves for which multiple comparisons were made.


1
0.5
0
S(t)
0
10
20
30
40
50
60
70
80
Number of days to colonization
1m
5m
15m
35m
60m
200m

Chapter 2: Part A

SEX AND SEASONAL DIFFERENCES IN TERRESTRIAL DISTRIBUTIONS AND CORE HABITAT ESTIMATES FOR POPULATIONS OF THE GRAY TREEFROG (*HYLA VERSICOLOR*)

Jarrett R. Johnson, Jason H. Knouft, and Raymond D. Semlitsch

ABSTRACT

Fragmentation of the terrestrial landscape has exacerbated the need to understand the core habitat requirements of mobile organisms, especially those that undergo seasonal migrations, such as pond breeding amphibians. Research indicates that pond-breeding amphibians spend much of their lives in terrestrial habitat at some distance from the aquatic sites on which they depend for reproduction. The core habitat for a particular individual encompasses the specific terrestrial habitat area adjacent to a breeding site that is used for activities such as foraging or overwintering, as well as the expanse of habitat through which it moved to reach the area. To elucidate core habitat requirements, gray treefrog movements through terrestrial habitat adjacent to breeding ponds were monitored using radiotelemetry and artificial arboreal refugia. Our results indicate that gray treefrogs undergo yearly migrations between foraging grounds, overwintering sites, and breeding ponds of 200 m or more, with a maximum recorded distance of 330 m. Furthermore, females travel further than males for reasons independent of body size,

placing them at the periphery of the core habitat of the population, and the distribution of males during the breeding season is biased towards breeding ponds relative to the non-breeding season. The data illustrate the importance of terrestrial habitat directly adjacent to breeding sites, and indicate that habitat loss resulting in small (<200 m radius) fragments of forested habitat may have a greater negative impact on females than males, and ultimately cause population declines.

INTRODUCTION

The importance of studies investigating the regional dynamics of populations increases as formerly continuous landscapes become fragmented by habitat destruction and alteration. Whereas the distributions of most organisms can be described as patchy, resulting from variation in biotic and abiotic factors (Andrewartha and Birch 1954), the term metapopulation specifically denotes the degree to which local populations are interconnected. The critical dynamics of metapopulations are the extinction and recolonization probabilities of each patch (Levins 1970; Hanski and Gilpin 1991; Hanski 1999), which vary based on spatial aspects of patches (e.g., size, quality, configuration), the composition of matrix habitat, and the movement behavior of the organisms under study (e.g., Fahrig and Merriam 1994; Kareiva and Wennergren 1995). In nature, metapopulations represent the midpoint of a gradient of interconnectedness that ranges from complete isolation to highly frequent inter-patch movements (Harrison 1991). However, populations are often assumed to interact as metapopulations, based on limited information regarding movement capabilities and habitat use or the absence of empirical evidence. Determining the relative scale of local patch and regional metapopulation dynamics will aid in identifying the most appropriate methods to preserve natural population processes and is a critical aspect of conservation biology as natural habitat is partitioned into preservation units.

Amphibians have been considered good candidates to exhibit metapopulation dynamics based on low movement capability (Sinsch 1990; Driscoll 1997), high rates of adult philopatry (Dole 1968; Gill 1978; Berven and Grudzien 1990; Sinsch and Seidel 1995), and for pond-breeding amphibians, localized activities within patches surrounding aquatic breeding sites (Marsh and Trenham 2001). However, these stereotypes of pond-breeding amphibians have been challenged (Marsh and Trenham 2001; Smith and Green 2005). Some studies have reported long-distance movements (i.e., >1 km; e.g., Carlson and Edenhamn 2000; Pilliod et al. 2002; Muths 2003; Bartelt et al. 2004) and a lesser degree of philopatry at single breeding ponds (at least for juveniles) than traditionally was believed (Jameson 1956; Gill 1978; Breden 1987; Berven and Grudzien 1990; Reading et al 1991; Sinsch 1992a; Sinsch 1997; Chapter 1). Some authors suggest that regional dynamics of amphibian populations are more similar to ‘patchy’ populations with a high degree of movement among sites, than metapopulations (Sinsch 1997; Marsh et al. 1999; Trenham et al. 2001). But the distinction between a ‘patchy’ population and a metapopulation is not strictly delineated, and numerous studies have determined that breeding site isolation influences patch occupancy rates (Laan and Verboom 1990; Sjögren-Gulve 1994). Furthermore, studies investigating the effects of matrix habitat composition and landscape features that constitute barriers to dispersal have determined that naturally high frequency of movements among sites can be severely altered by habitat fragmentation (Hecnar and M'Closkey 1996; Lehtinen et al. 1999; Joly et al. 2001) and urbanization (Fahrig et al. 1995; Vos and Chardon 1998; deMaynadier and Hunter 2000; Carr and Fahrig 2001; Pellet et al. 2004). Therefore, 'natural' amphibian population dynamics may be altered in the face of increasing habitat modification, and an improved understanding of the critical components of observed amphibian distributions will be beneficial to conservation efforts.

However, it is difficult to distinguish between movements at the local scale (i.e., seasonal migrations within populations) and those at the regional scale (i.e., permanent dispersal among populations) without explicit knowledge of local patch sizes. The extent of terrestrial habitat adjacent to breeding sites that supports a local population, and thus the scale of movements that are classified as 'migration' rather than 'dispersal', is poorly understood for amphibians. Delineation of local patch sizes is of further importance because other studies have found regional breeding pond occupancy rates of 100% for some pond-breeding amphibian systems (e.g., Gill 1978; Sinsch 1992b). High occupancy rates seem to be associated with rescue-effects in which a few 'source' patches produce dispersers that move to 'sink' patches and maintain local populations within sub-optimal sites (Brown and Kodric-Brown 1977; Pulliam 1988; Sinsch 1997; Carlson and Edenhamn 2000; Trenham et al. 2001). The factors that determine which patches will be 'sources' and which will be 'sinks' operate at the level of the local population, and depend on aspects of patch quality. The likelihood of a patch supporting a large number of individuals may depend on size and the relative impacts of terrestrial and aquatic habitats on patch carrying capacity, thus data regarding the extent of terrestrial habitat used by local populations of pond-breeding amphibians throughout the year are necessary before adequate strategies to preserve natural population dynamics in altered landscapes can be implemented (Halley et al. 1996; Drechsler and Wissel 1998; Snodgrass et al. 2000). Furthermore, understanding amphibian population dynamics are of critical importance considering the global phenomena of amphibian population declines, and the probability that terrestrial habitat destruction is among the primary causes (Houlahan et al. 2000; Stuart et al. 2004).

Pond-breeding amphibians rely on adequate terrestrial habitat for successful completion of various life history activities (Breckenridge and Tester 1961; Kramer 1973; Harris 1975; Semlitsch 1981; Freda and Gonzalez 1986; Denton and Beebee 1993; Kusano et al. 1995; Dodd 1996; Semlitsch 1998, 2000; Pope et al. 2000; Lamoureux et al. 2002; Bartelt et al. 2004). More and more data regarding appropriate protection requirements for terrestrial habitat is being produced in studies using various methods including mark-release/recapture and radiotelemetry. Recently, use of artificial arboreal refugia for capturing treefrogs has been successful in locating individuals in terrestrial habitats (Moulton et al. 1996; Boughton et al. 2000; Bartareau 2004). The use of artificial arboreal refugia to capture treefrogs is an important step in including arboreal species in the growing data set regarding the extent of terrestrial habitat use of pond-breeding amphibians. Furthermore, the highly mobile capabilities of treefrogs may provide core terrestrial habitat estimates that encompass many other species of amphibians that breed at the same sites.

In our study, we combine the use of artificial arboreal refugia and radiotracking to determine core habitat requirements of the gray treefrog (*Hyla versicolor*) and identify previously unreported aspects of gray treefrog life cycle. Specifically, we determine terrestrial distributions of adult gray treefrogs during the breeding and non-breeding seasons, and evaluate hypotheses regarding differential habitat-use based on sex and size. Lastly, we discuss the implications of reductions in habitat patch size on amphibian populations, and provide recommendations regarding protection of terrestrial habitat surrounding pond-breeding amphibian breeding sites.

METHODS

Mark-release/recapture

Beginning the fall of 2002 and continuing through the fall of 2004, we monitored three gray treefrog breeding sites located within the Thomas Baskett Wildlife Research Area near Ashland, Boone County, Missouri. Treefrogs were captured in artificial arboreal refugia with an upper opening 3 m above the ground. Each arboreal refuge consists of 60 cm long sections of 3.8 cm inside diameter, black acrylonitrile-butadiene-styrene pipe attached to trees with bungee cords (Appendix A). Refugia are sealed at the bottom to allow rainwater to fill to 15 cm deep. Water levels remain constant during monitoring because an inside pipe sits loosely within the bottom capped portion (Appendix A). Frogs may enter and leave the pipe-trap freely at the upper opening.

Refugia were placed on large diameter deciduous trees and arranged into seven transects. Each transect extended from a breeding site into secondary growth (~100 yr) oak/hickory (*Quercus spp./Carya spp.*) forest with sugar maple (*Acer saccharum*) understory at intervals of 1, 5, 15, 35, 60, 100, and 200 m. We placed two pipes at each of the seven sampling intervals, in each of the seven transects, for a total of 98 artificial arboreal refugia that were checked during daylight hours for the presence of treefrogs. If present, frogs were extracted from the pipes with a sponge, as described by Boughton et al. (2000). We measured snout-vent length with a plastic ruler for all individuals at least once per season, and body mass with a Pesola spring scale starting in the spring of 2004. If not previously marked at breeding sites (see below), toes were excised to produce a unique toe-clip identification. After processing, frogs were returned to the pipes, which were then placed back on the tree. Additionally, we performed nocturnal monitoring of the three aquatic breeding sites to verify that individuals captured in artificial refugia were members of the local breeding population. At the ponds, individuals were captured by hand and processed as described above for the artificial refugia.

Radiotelemetry

During the post-breeding season adult emigrations (from July to October) of 2003 and 2004, a subset of individuals captured in refugia were implanted with radiotransmitters and tracked to their overwintering sites. We conducted the radiotelemetry portion of this study to evaluate the accuracy of arboreal retreat captures to estimate final position in the terrestrial environment. In other words, we wanted to determine if captures in refugia were occurring on foraging grounds that were distinct from overwintering sites, and skewing our interpretation of observed ultimate terrestrial distributions of individuals. We selected 22 adult (10 male and 12 female) gray treefrogs captured in arboreal retreats to be implanted with radiotransmitters (Appendix B). We selected individuals based on capture date (approx. 25 d prior to the first freezing temperature) and body mass. We used 0.85 g BD2 radiotransmitters (14 x 6.5 x 3.5 mm) with internal helical antennae from Holohil Systems Ltd. (Carp, Ontario, Canada K0A 1L0) that had an expected battery life of at least 25 days. Transmitters did not exceed 10% of an individual's mass (Richards et al. 1994), thus only frogs weighing greater than 8.5 g were included in the study. Within 24 hours following implantation (Appendix B for detailed procedures), individuals were released at the point of capture and tracked for the duration of the transmitter battery life. Prior to release, the behavior (i.e., body posture, avoidance behavior) of each animal had returned to normal (Appendix B). Summaries of maximum distance from breeding ponds and the population space-use estimate include only individuals captured previously at breeding ponds. Population-level kernal density space-use estimates were generated using the animal movements extension in ArcView. Transmitters were removed following tracking and animals replaced at their overwintering locations.

Simulation of random distribution

Simulated dispersal events were generated to assess whether treefrog dispersal patterns from the mark-recapture study were random or directional after individuals left breeding ponds. The mean and standard deviation of individual daily movements were calculated from the radiotelemetry data. Using these values to parameterize the simulation, 300 "individuals" were allowed to disperse randomly from the center of a "pond." Each individual was allowed to move in any direction each day and the distance moved was randomly chosen from a normal distribution with a mean and standard deviation identical to that calculated from the telemetry movement data. The simulated movements were terminated when the mean distance from the pond of simulated individuals was equal to that of the observed data from individuals captured in artificial arboreal refugia.

Upon termination of the simulation, the number of individuals at each sampling distance (1, 5, 15, 35, 60, 100, and 200 m) was recorded. Because equal numbers of pipes were used to recapture individuals at different distances during the mark-recapture study, appropriate sampling intensity was applied to the simulated data at each distance. For example, the circumference at 100 m from the pond edge is 15.1 times greater than it is 1 m from the pond edge. Consequently, only 6.6% of the simulated individuals at 100 m were considered as recaptures relative to the simulated recaptures at 1 m. The percent of total recaptures at each sampling distance was calculated from these data. The simulation and sampling processes were repeated 1000 times and a mean percentage of recaptures was calculated for each distance. We then compared the simulated frequency distribution to the observed number of captures at each distance using Kolmogorov-Smirnov (KS) tests in SPSS to determine if the distribution of movements from the mark-recapture data were different than expected from a Brownian motion (i.e., random) model of dispersal.

We also used SPSS to perform KS tests to compare observed distributions of total captures and unique captures of males and females during both the breeding and non-breeding seasons. Additionally, we compared mean average distance from breeding ponds for males and females and with a two-tailed T-test, and performed simple linear regression to relate SVL, mass, and length-specific mass (mg/mm) to distance.

RESULTS

Mark-release/recapture

During the breeding seasons of 2003 and 2004 we marked 651 adult (577 male and 74 female) gray treefrogs and recorded a total of 700 recaptures (1351 total captures) at three breeding ponds. Of these individuals, only 33 (5.1%) were recaptured in artificial refugia. Combined with treefrogs never captured at breeding ponds (i.e., caught in refugia only), we captured a total of 109 adult frogs in artificial refugia and recorded 595 recaptures. The sex ratio of individuals captured in artificial refugia was 42:67 (F:M), with males representing 61.5% of individuals captured. Twenty-six individuals (18 males, 8 females) were captured only once in refugia and were considered to be migrating to locations unknown. Forty-three individuals were captured and marked as juveniles during the course of the study, with 11 maturing into males and 5 maturing into females. We did not include juveniles in any of the analyses.

We combined the captures recorded in each of the paired artificial refugia at each sampling distance within transects due to high frequencies of movements among them. When we partitioned the activity period of treefrogs into 4 seasons: after overwintering and before chorus formation (pre-breeding season), during chorusing (breeding season), after chorusing had ceased and before overwintering (post-breeding season), and the period in which frogs were absent from refugia (overwintering season), we found that eighty individuals exhibited foraging site fidelity and returned to the same artificial refuge during at least two seasons. Excluding the overwintering season, some individuals used the same refuge for 6 consecutive seasons. If we consider only treefrogs that were captured on multiple occasions and exclude frogs captured only during the final major monitoring season of the study (Summer 2004), 98.8% of individuals were philopatric to non-breeding habitat. We considered treefrogs to be philopatric to arboreal refugia if individuals were captured and subsequently recaptured at the same refuge location separated by a prolonged (i.e. several months) absence for overwintering or temporary absence (i.e. several weeks for males, and several days for females) during the breeding season.

The overall distribution of captures was not significantly different from an even distribution (i.e., equal number of captures) throughout the seven distances (Fig. 1), when considering either total captures (D=0.753, P=0.621), and when omitting recaptures (i.e., unique captures; D=0.532, P=0.940). However, when total capture data were separated for males and females, we found that females were distributed at further distances, and males were located nearer to ponds (D=2.907, P<0.001; Fig. 2). Additionally, the average distance from breeding ponds was significantly different for males than for females for both total captures ($T_{2\text{-tail}}$=1.966, df=407, P<0.001), and unique captures ($T_{2\text{-tail}}$=1.988, df=85, P=0.004; Fig. 3).

During breeding seasons we recorded 146 total captures and 60 individuals in arboreal refugia. The overall distribution of these captures encompasses the same range as that of combined yearly data. However, when plotting females versus males, we found that while the distribution of females during the breeding season was not different from the non-breeding season (D=0.923, P=0.362; Fig. 4A), the distribution of males was more restricted towards the pond during the breeding season than during the non-breeding season (D=1.843, P=0.002; Fig. 4B). Overall, neither SVL ($F_{1,47}$=0.003, P=0.958) nor mass ($F_{1,47}$=1.245, P=0.270) of treefrogs appeared to have an effect on distance from breeding sites for adults captured multiple times. Length specific mass (mg/mm) was more associated with distance from ponds than either mass or length alone, but was still insignificant ($F=_{1,47}2.17$, $P=0.147$; Fig. 5). Males and females differed significantly in size (mg/mm; $T_{2\text{-tail}}=9.4$, $df=25$, $P<0.001$). However, we found no effect of size on capture distance within males ($F_{1,28}<0.001$, $P=0.990$) or females ($F_{1,17}=1.670$, $P=0.214$; Fig. 5).

Radiotelemetry

Two of the 22 individuals implanted with radiotransmitters were omitted from the analyses due to low numbers of relocations ($N=1$ or 2). We relocated the remaining 20 individuals an average of 24 times (± 0.9 SE) each, for a total of 485 relocation points. The average maximum distance from breeding sites was 172.4 m (±28.8 SE), and was not significantly different than the average distance of overwintering sites from breeding sites (169.6 ±28.5 SE; $T_{2\text{-tail}}=2.02$, $df=38$, $P=0.95$). The maximum distance traveled by any treefrog for which the breeding location was known was 270.9 m, while 331.9 m was the maximum distance from a breeding site that was reached by any treefrog originally captured within the artificial refugia arrays. One pair of space-use estimates overlapped within a year, and four overlapped among the two years of the study, but no radiotracked animals were ever located in the same tree. For detailed information regarding individual space-use estimates and overwintering sites, see Johnson and Mahan (Chapter 2B). The population-level space-use estimate for the breeding pond at which most animals were tracked, extends 340 m and illustrates the extent of terrestrial habitat use adjacent to breeding ponds (Fig. 6). Treefrogs that were nearer to adjacent breeding sites were excluded from the population-level space-use diagram but were included in the calculation of the mean distance traveled.

Simulation of random distribution

The distribution of computer generated 'individuals' following simulated random movements, and application of capture probabilities that approximated our sampling scheme, was significantly different from the observed distribution of total captured individuals (D=5.008, P<0.001; Fig. 1). Recaptured individuals were found at a higher frequency at greater distances than expected from the random-walk simulation data, indicating directional movement away from the pond by marked individuals.

DISCUSSION

Core terrestrial habitat

The delineation of habitat that encompasses local populations is a necessary component of any attempt to understand the interactions among populations in a landscape-level context. We use the term 'core habitat' to describe the terrestrial habitat that includes the extent of seasonal migrations that are important to local population dynamics (Semlitsch and Jensen 2001; Semlitsch and Bodie 2003). Conversely, dispersal movements occurring beyond the boundaries of the local habitat patch (i.e., not contained within the core habitat) are more important to regional-level dynamics, and are beyond the scope of this study. In continuous habitat, the boundaries of the core habitat of a population may be important only to distinguish between within- and among-patch dynamics, but in fragmented landscapes core habitat boundaries can be used to determine the suitability of existing patches (i.e., fragments) and the appropriate scale at which to preserve terrestrial habitat.

Based on captures within artificial arboreal refugia, we found that adult gray treefrogs were evenly distributed throughout the terrestrial habitat surrounding aquatic breeding sites up to at least 200 m (Fig. 1). However, males and females did not exhibit the same distributional patterns across this range. Radiotelemetry data illustrate that individuals may move even further (e.g., 270.9 m) into forested terrestrial habitat during non-breeding seasons to reach preferred foraging and overwintering locations. These distances are greater than those observed by Roble (1979), who recorded post-breeding season movements of juvenile gray treefrogs only within 125 m from breeding ponds, and less than the 630 m within-season interpond movements recorded by Ritke et al. (1991). We do not contend to have determined precisely the extreme tail of the treefrog migratory distribution, but rather to have illustrated that terrestrial habitat-use by gray treefrogs is extensive and non-randomly distributed.

We suggest that gray treefrog terrestrial habitat-use continues beyond the 200 m in which our artificial refugia were placed, and core habitat extends at least 270 m from breeding sites, based on the maximum migration recorded by radiotelemetry. In Figure 5, we outline the boundaries of the radiotracked population at one of our field sites, based on 95% and 50% kernal space-use estimates. The observed population-level space-use estimate reflects the location of the artificial refugia from which the sample of radiotracked individuals was drawn. We did not track, nor sample treefrogs in the fields east of the ponds. The space-use estimate was used to generate the concentric circles denoting our core habitat estimates as if habitat surrounding the pond was uniform.

Within our study area, the average distance between clusters of breeding sites was 491.8 m (±48.8 SE; Fig. 6). Therefore, core habitat radii extending towards adjacent breeding pond clusters overlap, and individuals found in close proximity during non-breeding seasons may belong to separate local populations. Furthermore, if post-metamorphic subadults are the primary dispersal agents, movements among sites should benefit from a lack of inhospitable matrix. When the 'boundaries' of local habitat patches overlap, and dispersal events are common, the use of 'patchy' population dynamic models in which each local population does not experience a high extinction risk relative to the entire array of patches, may best approximate the interactions among amphibian breeding populations. Our results are similar to those of Carlson and Edenhamn (2000) for *H. arborea*, in which an average interpond distance of 441 m resulted in a system of ponds that was unaffected by local extinctions, due to immediate recolonizations. What remains unclear, are the specific effects of interruptions of continuous habitat such as roads, pastures or agricultural fields, on the local dynamics occurring within core habitat. In a fragmented landscape, movement among sites may be severely inhibited (e.g., Rittenhouse and Semlitsch *in review*), and metapopulation dynamics more prevalent than indicated by our data.

The factors that drive the observed even distribution are unclear. Rarely were multiple frogs captured in a single artificial arboreal retreat simultaneously, and while space-use estimates varied greatly, most were fairly restricted overall. The low frequency of multiple captures per pipe, and lack of large overlaps in space-use estimates suggest that the even dispersion of individuals may be due to competitive interactions involving territoriality and defense of foraging sites. Studies of competitive interactions during the non-breeding season are rare, but Buchanan (1988) found some evidence that *Hyla squirella* defended artificial arboreal refugia constructed from bamboo in experimental trials. Competitive interactions regarding arboreal refugia may be important in determining core habitat estimates, because higher population sizes would require higher quality sites (i.e., high density of refugia), or greater patch areas if the abundance of resources were uniform. Marsh (2000) and Stewart and Pough (1983) each found that by artificially increasing pond density and retreat density, respectively, that frog populations increased.

Furthermore, the high degree of foraging site fidelity, and the significant difference between the observed and simulated distribution of random movements away from breeding sites, indicates that individual movements are directed and meaningful. Non-breeding season site fidelity has been anecdotally observed for other anurans (Goin and Goin 1957; Neill 1957; Kelleher and Tester 1969; Crump 1986), and further illustrates the importance of terrestrial habitat resources for amphibians.

Differences among males and females

Sexual dimorphism is prevalent throughout nature, and can either be the result of sexual selection or niche divergence (Darwin 1874; Shine 1989). When the size of females exceeds that of males, ecological causes such as resource competition, resulting in niche divergence have been used to explain the differences between the sexes (Shine 1989). Furthermore, differing aspects of reproductive biology (i.e., energetic costs associated with gamete production, benefits resulting from [or the effects of] increased gamete production, breeding behavior) may lead to changes in the location of optimal foraging habitat that maintains body sizes that maximize reproductive fitness for each sex. Determining if ecological differences between the sexes are the causes or the result of sexual dimorphism is difficult, and depends on whether the observed differences are adaptive (Shine 1989).

We found that the male size was significantly smaller than female size, and locations of males and females within the habitat adjacent to breeding sites differed, with females traveling further away from ponds than males (Fig. 3). Females have been found to migrate longer distances than males in other anurans (Pilliod 2002; Muths 2003; Bartelt 2004), and this behavior may be attributable to a variety of reasons. Females may travel further to reach foraging locations that have higher densities of prey, or more preferred prey species, to maximize energetic resources that can be used to increase egg number, egg quality, or clutch frequency. But, if the distribution of insect prey is uniform, females may be seeking locations with reduced densities of conspecific competitors, to increase the relative abundance of prey available to them. Furthermore, females generally arrive at breeding sites after males have established choruses, and Sinsch (1992a) found that females are less philopatric to breeding sites than males. Females may benefit from the opportunity to choose a breeding site based on male chorus characteristics, and by positioning themselves at greater distances from breeding sites, they may be in a better position to assess multiple breeding choruses. Males may benefit from traveling less and the ability to reach a breeding site quickly to acquire a desirable breeding territory and opportunities to encounter more females (Fellers 1979).

The observed differences between the distributions of males and females do not appear to be the result of size differences alone. Although the length specific mass (mg/mm) of females was significantly larger than males, no overall relationship among capture location and size was detected for either males or females (Fig. 5). This indicates that size-independent factors are driving females to move farther than males, rather than morphological limitations of males, and suggests that niche divergence has adaptive significance in this species.

Regardless of the underlying cause of the distributions, the result is that females are on the periphery of the population and exist along the border of the core habitat patch. Thus, reduction in patch size because of land use will have severe impacts on the probability of population persistence by reducing the number of individuals that a patch can support, but also by eliminating a higher proportion of females than males. In sexual species, the capacity of a population to recover following population reduction is highly dependent on the number of females, because a single male can fertilize many clutches of eggs. As the percentage of core habitat protected decreases, the proportion of females affected increases more rapidly than that of males, and the overall effective population size decreases dramatically. For example, removal of the 200 m experimental arboreal refugia in our study results in a 51.5% loss of females, but only a 13.2% loss of males captured in this study. The percentage of females lost from such a 50% reduction in patch size is most likely a very conservative estimate due to our sampling scheme, as we only have one sampling location past 100 m from breeding sites. Nonetheless, even this conservative estimate of the percentage of females affected by habitat loss could have negative impacts on local population dynamics given the high mortality rates of eggs, tadpoles, and juveniles, and regional dynamics by reducing the number of post-metamorphic dispersers. Further investigation regarding the mechanisms underlying niche separation and sexual dimorphism in pond-breeding amphibians could provide necessary information regarding the importance of conserving habitat adjacent to breeding sites that encompasses the natural range of migratory movements.

Variation between seasons

Similar to many species of amphibians, gray treefrogs are classified as prolonged-breeders and typically breed over several months. Past research has suggested that breeding chorus tenure affects the reproductive success of individuals, such that males spending more time calling at breeding sites mate more frequently than males that are absent or not actively calling (Kluge 1981; Godwin and Roble 1983; Gerhardt et al. 1987; Ritke and Semlitsch 1991; Sullivan and Hinshaw 1992; Murphy 1994a). However, calling is energetically costly and even the 'best' males must leave the chorus occasionally to forage (Murphy 1994b). What was not known was how far males ventured into surrounding terrestrial habitat to acquire food resources and replenish energetic reserves.

We found that the distribution of males in arboreal retreat sites during the breeding season (i.e., while other males are calling at breeding sites) was significantly constricted towards the pond relative to the distribution of males during the non-breeding seasons (Fig. 4B). For males, the average distance to breeding sites was shorter during breeding seasons than during non-breeding seasons, indicating that some males do not move as far to replenish energy reserves in between calling bouts as they do after the breeding season is finished. Therefore, maintaining appropriate terrestrial habitat adjacent to breeding sites may be particularly important to the duration of the breeding season, chorus tenure, and breeding success by allowing only short absences of males from choruses.

In contrast, females spend very little time at breeding ponds during any portion of the year, usually no more than 48 hrs and maintain a relatively constant distribution surrounding breeding sites (Fig. 4A). When females do migrate to breeding sites, the trips last just long enough to breed (Ritke et al. 1990; Sullivan and Hinshaw 1992) and return to their terrestrial foraging grounds. For example, we found one female in our study (#0769) that was observed in a 100 m artificial arboreal retreat during the day, then in amplexus at a breeding pond the following night, and then back in the same arboreal retreat the next day.

While males rely on habitat immediately adjacent to breeding ponds during the breeding season, females rely on more distant habitat. If terrestrial habitat adjacent to ponds was lost, it may be true that males would simply move further from breeding ponds because some short distance (~60 m) movements of adults through non-forest habitat has been observed in other studies (Chapter 3). However, what remains to be determined are the consequences of overlapping male and female distributions in habitat fragments that are too small to support niche separation. In non-altered habitats, the absence of conspecific male competitors at distant foraging sites could allow females to forage more effectively and produce higher quality eggs, or produce multiple clutches during the breeding season (Ritke et al. 1990). Ultimately, the maintenance of continuous terrestrial habitat appropriate for amphibians or habitat complementation (Dunning et al. 1992) protects natural processes beyond those that can be measured by the movements of individuals alone.

Conservation Implications

Recently, biologists have recognized the importance of upland terrestrial habitat surrounding wetlands on the persistence of amphibian populations. Investigations regarding terrestrial habitat movements have illustrated that current buffer zone guidelines for wetlands do not contain the extent of terrestrial habitat utilized by amphibians during the non-breeding season (Semlitsch 1998; Semlitsch and Bodie 2003).

The use of core habitat estimates that include a large percentage of the movements of individuals during the non-breeding season is an appropriate metric upon which to base decisions regarding amphibian habitat protection requirements. Further, if the goal is to maintain natural amphibian population dynamics, buffer zones could be used beyond the perimeter of core habitat estimates to protect the life history activities of individuals along the periphery from edge effects (Murcia 1995). We suggest that protection of terrestrial habitat adjacent to treefrog breeding sites extend greater than a 100 m radius to encompass 50% of the total population, and greater than 300 m to include 95% of the total local population. Our results indicate that non-breeding season movements extend further from breeding ponds than the movements associated with reproduction (Chapter 1). However, application of these core habitat estimates to other populations may require information on population size, due to the observed even distribution of captures. Also, when extending these values to other species we caution that information regarding niche separation of males and females is required to adequately determine the relative impact of land use on effective population size and cumulative reproductive fitness if less than 95% of the habitat used by amphibian populations is protected.

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Figure 1. Average percentage of total recaptures in artificial arboreal refugia at each sampling interval compared to 1000 iterations of simulated distributions comprised of random-walk movements.

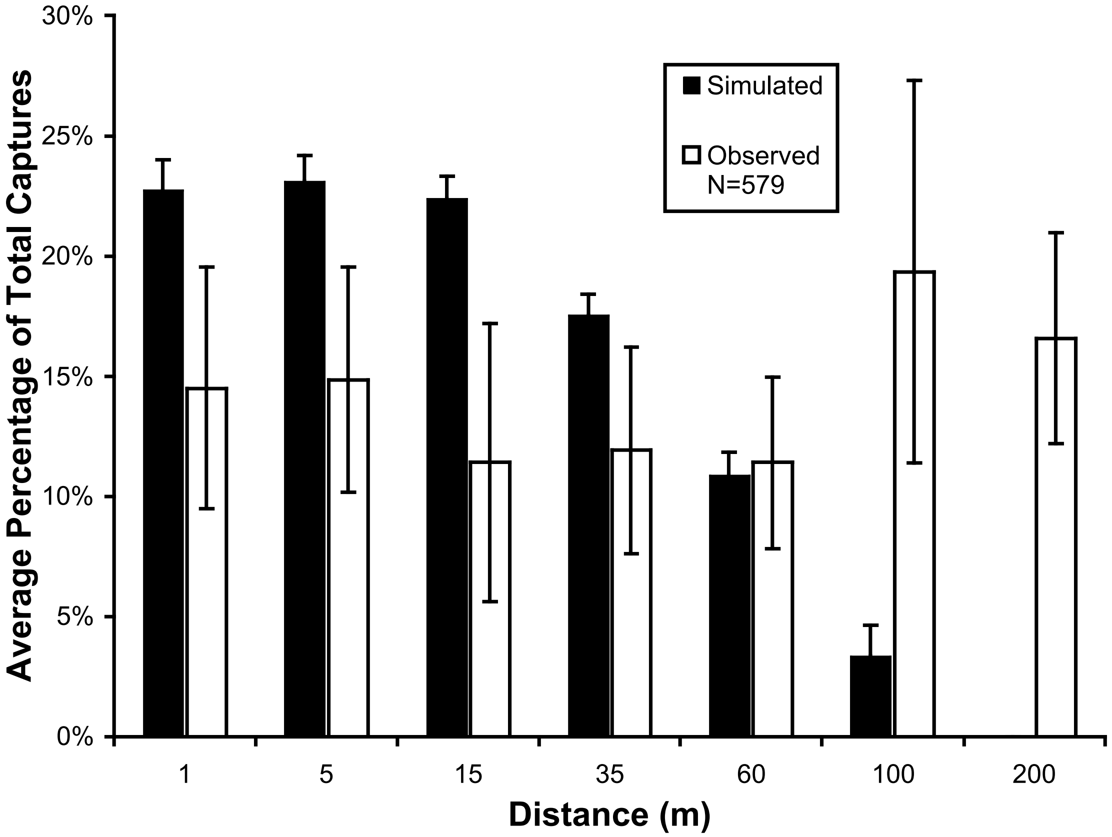
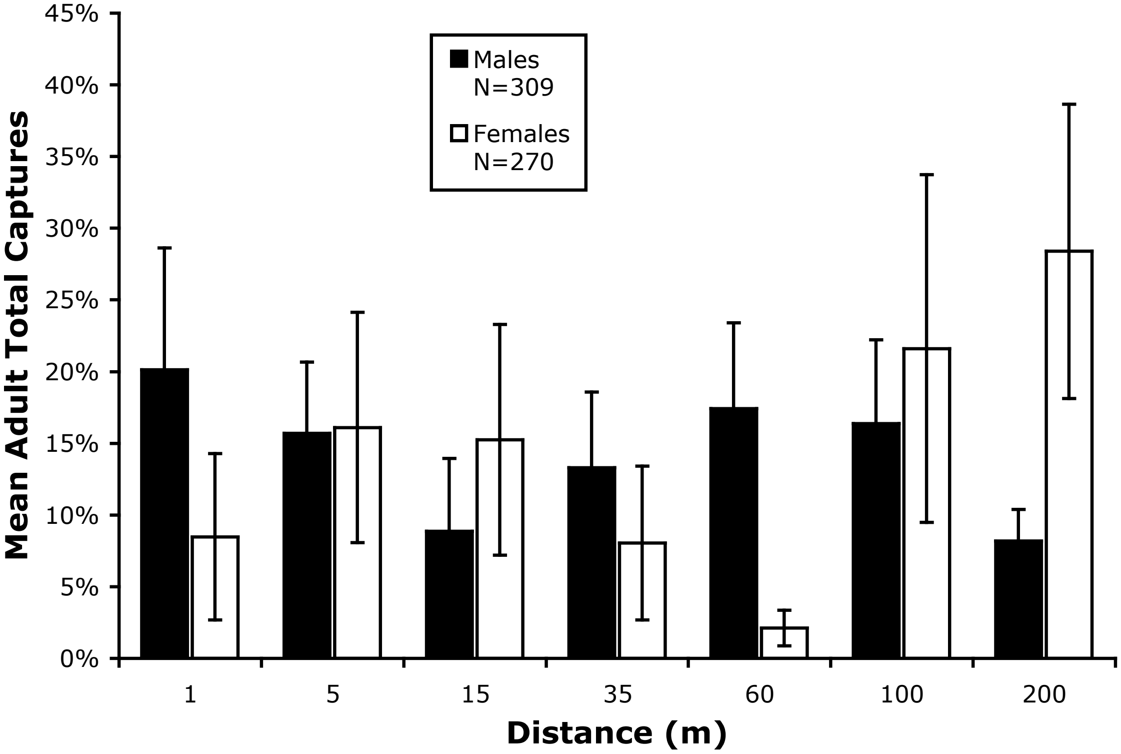
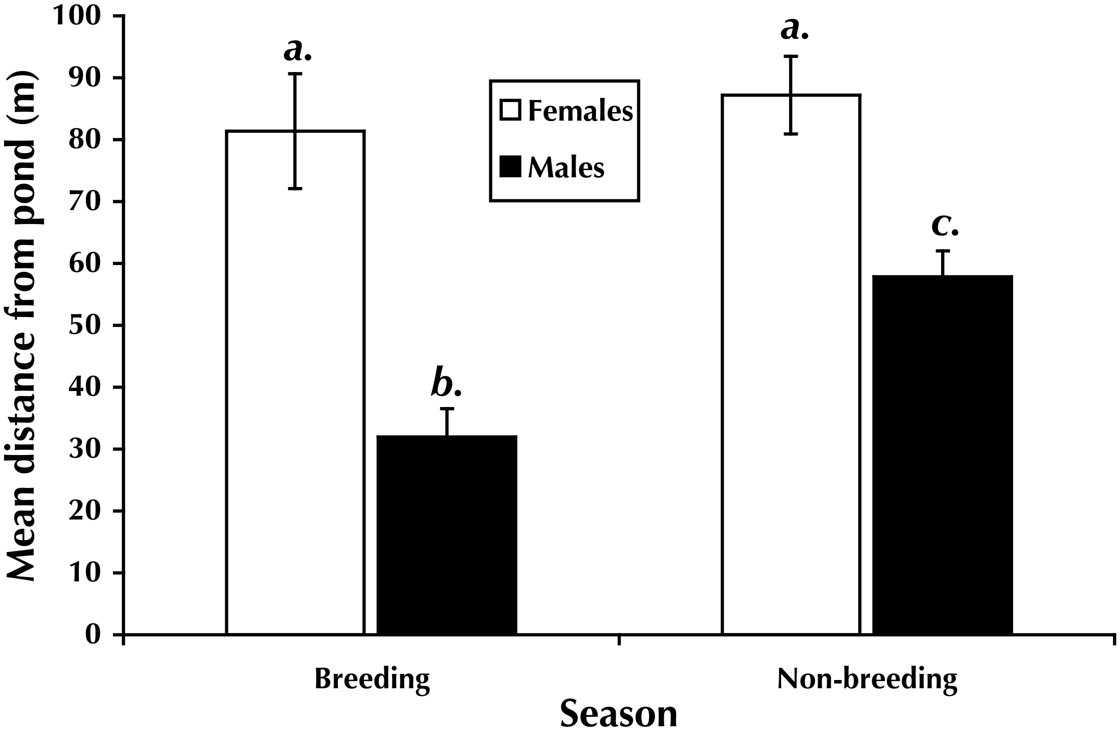

30%
25%
20%
15%
10%
5%
0%
Average Percentage of Total Captures
■ Simulated
□ Observed
N=579
1
5
15
35
60
100
200
Distance (m)

Figure 2. Comparison of the average percentage of total male and female recaptures in artificial arboreal refugia at each sampling interval.



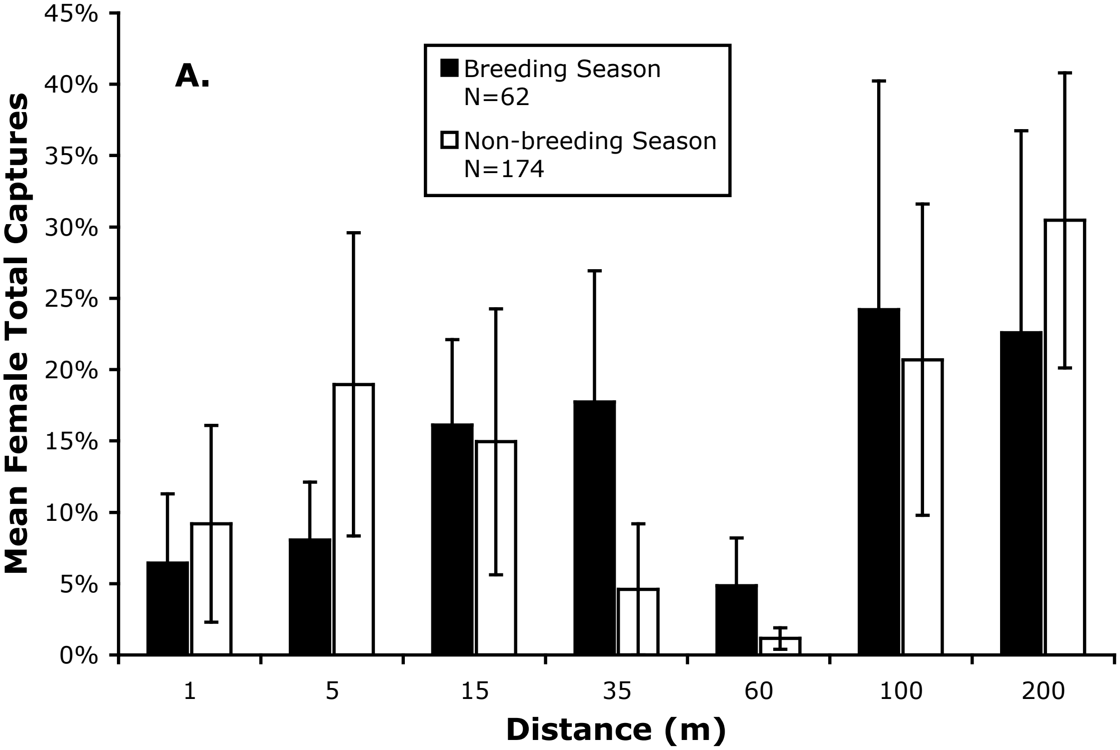
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
Mean Adult Total Captures
■ Males
N=309
□ Females
N=270
1
5
15
35
60
100
200
Distance (m)

Figure 3. Comparison of male and female average distance from breeding sites during the breeding and non-breeding seasons based on captures in artificial arboreal refugia.



100
90
80
70
60
50
40
30
20
10
0
Mean distance from pond (m)
a.
b.
a.
c.
☐ Females
■ Males
Breeding
Non-breeding
Season

Figure 4. Average percent abundance of total recaptures during the breeding and non-breeding seasons for **A)** females and **B)** males.


A.
■ Breeding Season N=62
□ Non-breeding Season N=174
Mean Female Total Captures
0%
5%
10%
15%
20%
25%
30%
35%
40%
45%
1
5
15
35
60
100
200
Distance (m)

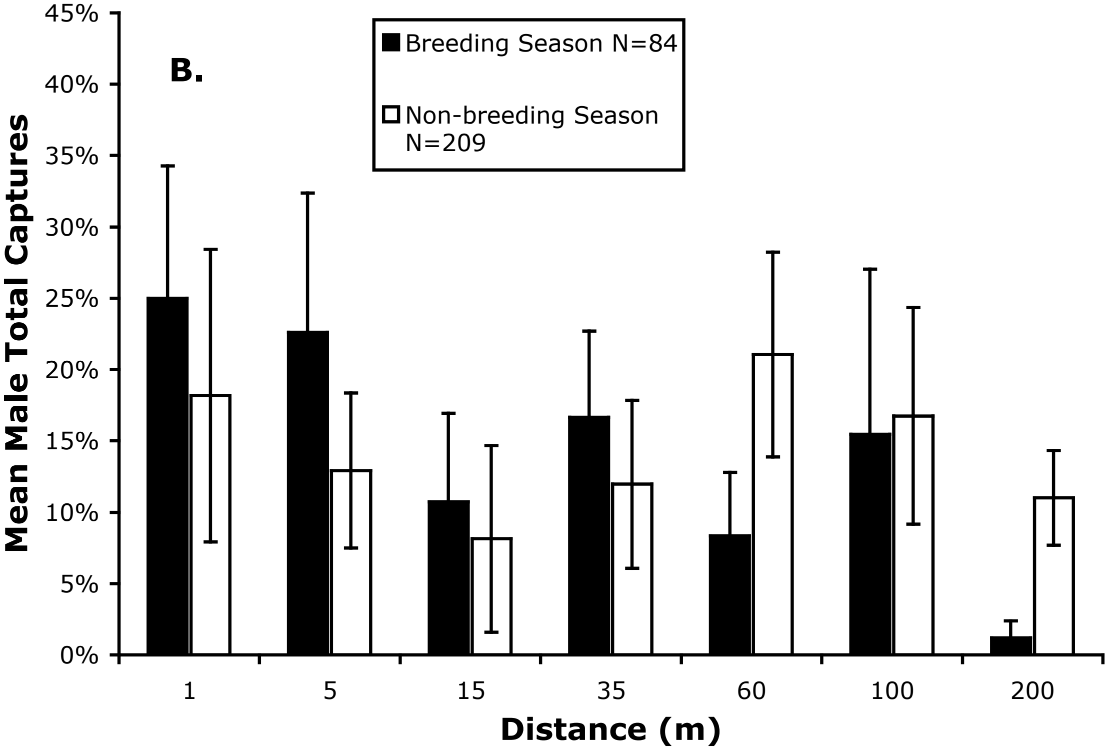

B.
■ Breeding Season N=84
□ Non-breeding Season N=209
Mean Male Total Captures
0%
5%
10%
15%
20%
25%
30%
35%
40%
45%
1
5
15
35
60
100
200
Distance (m)

Figure 5. Scatter-plot and best-fit line for the effect of length specific mass (mg/mm) on capture distance for males and females.

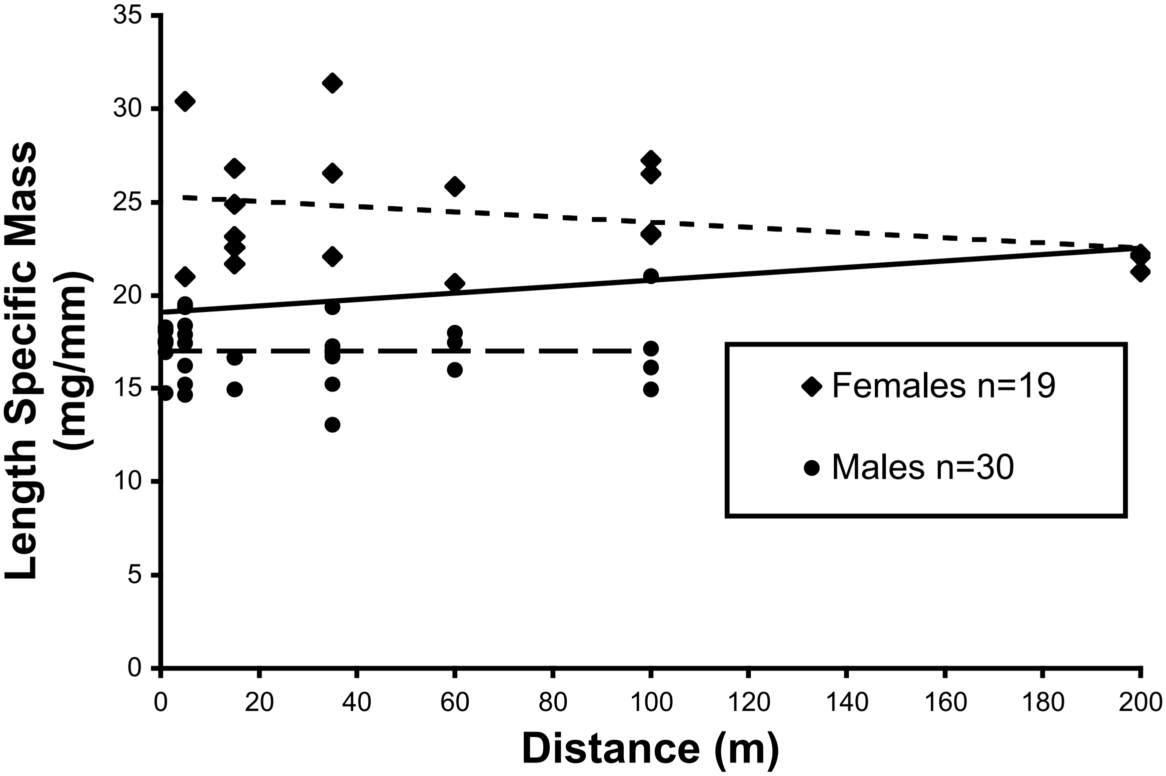

Length Specific Mass (mg/mm)
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
140
160
180
200
Distance (m)
◆ Females n=19
● Males n=30

Figure 6. Aerial photograph of study site, illustrating distance among breeding sites, proposed core habitat area (300 m and 100 m radii circles), and population-level space-use estimate (hatched area; outer: 95% kernal estimate and inner: 50% kernal estimate).


N
W
E
S
0
55
110
220
Meters

Chapter 2: Part B

BIOTIC AND ABIOTIC FACTORS AFFECTING TERRESTRIAL MICROHABITAT USE BY GRAY TREEFROGS (*HYLA VERSICOLOR*): EVIDENCE FROM ARTIFICIAL ARBOREAL RETREATS AND RADIOTRACKING

Jarrett R. Johnson and R. D. Mahan

ABSTRACT

Estimates of habitat patch quality are largely overlooked in studies of metapopulation dynamics in fragmented landscapes. When included in metapopulation models, patch quality is estimated by patch size alone and there is generally a poor understanding of the important habitat aspects upon which to base biological estimates of patch quality. Our study identifies meaningful features of terrestrial habitat for the gray treefrog, (*Hyla versicolor*) using mark-recapture/release techniques in artificial arboreal refugia and radiotracking. We found that maximum temperature, minimum humidity and previous three days rainfall total were the most important weather variables in determining the frequency of captures in artificial refugia. High humidity and rainfall decreased captures, while high temperatures increased the number of treefrogs captures in artificial refugia. Environmental variables similarly affected microhabitat use of individuals during the radiotracking portion of the study. Refugia captures were also affected by both the species of tree and the diameter of tree in which refugia were placed. Refugia placed in large trees tallied more captures than those in smaller trees, and refugia in white oaks (*Quercus alba*) captured individuals more frequently than expected at random. The use of artificial refugia benefited frogs by limiting the rate of evaporative water loss relative to individuals placed in screen replicas of artificial refugia. We found that non-breeding season space-use estimates encompassing overwintering and foraging locations of gray treefrogs are relatively small, and may include only a few adjacent trees. We suggest that aspects of patch quality may be important in determining the persistence of local populations of gray treefrogs. Finally, we discuss the effect of alteration to critical features of gray treefrog terrestrial habitat on regional population dynamics.

INTRODUCTION

The relative importance of local (i.e., within habitat patch) dynamics versus landscape-level dynamics is a central component of attempts to predict the effects of fragmentation on populations. At the landscape scale, maintaining connections among populations has been shown to be important in determining species richness within patches and patterns of occupancy among patches (e.g., Laan and Verboom 1990; Hecnar and M'Closkey 1996; Lehtinen et al. 1999), but the habitat that supports local populations is also critical to regional persistence (Halley et al. 1996; Drechsler and Wissel 1998), and the local scale should not be overlooked. Much metapopulation research has focused on the effects of patch size and patch isolation on regional patch occupancy patterns and local extinction probabilities (e.g., Hanski 1998; Moilanen et al. 1998; Moilanen 1999). These studies illustrate that fragmentation results in decreased overall dispersal among sites, and increased local and regional extinction probabilities, with smaller patches being at higher risk than larger patches.

Few attempts have been made to quantify biological aspects of patches (i.e., factors other than size) that may affect the persistence of local populations that have become isolated by fragmentation. Studies that have incorporated patch quality estimates into metapopulation models have assumed a general relationship between large patches, high population sizes, and decreased extinction probabilities (Hanski et al. 1995; Drechsler and Wissel 1998; Forys and Humphreys 1999). And in some instances, aspects of patch quality did not significantly improve metapopulation models relative to models with estimates of size and isolation alone (e.g., Moilanen and Hanski 1998). More recently however, researchers have suggested that use of environmentally-based patch quality estimates do improve our understanding of the effects of fragmentation on natural populations (Thomas et al. 2001; Fleishman et al. 2002; Franken and Hik 2004). Therefore, conservation biologists require information regarding the characteristics of local habitat patches that are important to sustaining healthy subpopulations (i.e., those that produce dispersers and can maintain connections among sites).

Assessment of biological aspects of patch quality requires detailed investigations for particular taxa, and specific knowledge of the distribution of resources. For example, if resources are uniformly distributed and density-dependence or territoriality influences population size, the general relationship between patch size and patch quality should hold, and large habitat patches should support larger populations. On the other hand, if resources are clumped, or if high local densities do not affect individuals, patch size would not explicitly determine patch quality. Irrespective of patch size, high quality patches should support greater population sizes. Larger populations have the capacity to produce more dispersers, and act as source populations that can ‘rescue’ populations in lower quality sites from local extinction. Source-sink or ‘rescue’ dynamics have been identified as an important aspect of the landscape level processes of many pond-breeding amphibians (Gill 1978; Sinsch 1992; Trenham et al. 2001). Information regarding the habitat variables that determine patch quality will help to distinguish sources from sinks. The ability to identify source populations, or the potential for a patch to become a source population, could help to prioritize conservation efforts, and reveal some of the mechanisms that are contributing to worldwide amphibian population declines (Houlahan 2000; Stuart et al. 2004).

The avoidance of aquatic habitat with fish or other predators constrains many amphibians to utilize highly ephemeral breeding ponds for larval development. As a result, early studies of pond-breeding amphibian population regulation focused solely on factors affecting the quality of aquatic habitat (i.e., hydroperiod, predator/competitor/prey guilds, etc. [e.g., Wilbur 1980]). The variable environment (i.e., hydroperiod) of amphibian breeding sites, also leads to naturally high probabilities of local extinction (Drechsler and Wissel 1998), and in the absence of colonization from more productive sites, the accumulation of local extinctions should cause the entire system of populations to go extinct. However, pond occupancy rates of 100% are not uncommon for amphibians (Gill 1978; Sinsch 1992; Carlson and Edenhamn 2000; Trenham et al. 2001), and local extinctions are rapidly offset by dispersal among populations (Hecnar and M’Closkey 1996; Lehtinen and Galatowitsch 2001; Pechmann et al. 2001), which is not unexpected for organisms that have evolved within highly temporally variable habitats

(Hanski 2000; Marsh 2001). Similarly, amphibians that are long-lived have the capacity to forego breeding in years in which conditions are unfavorable and populations are thus capable of sustaining several years of complete reproductive failure (Semlitsch et al. 1996). Therefore, long-term regional amphibian persistence in the absence of inter-pond movements may depend on the characteristics of sites that support large populations (Halley 1996; Drechsler and Wissel 1998), or allow adults to persist in the terrestrial habitat when reproduction is not possible.

Over the past decade, studies investigating the terrestrial habitat use of pond-breeding amphibians have illustrated that terrestrial habitat adjacent to breeding sites is used extensively for a variety of life-history activities (e.g., Semlitsch 1998, 2000; Semlitsch and Bodie 2003). Gaining an understanding of the environmental factors that influence the behavior of individuals is critical to the development of models that predict the effects of changes to terrestrial habitat features on amphibian populations. While it may seem intuitive that terrestrial habitat has importance, little information exists regarding the fate of individuals (and ultimately the population) during the non-breeding season. The paucity of information on terrestrial habitat use of pond-breeding amphibians stems from the difficulty in capturing individuals as distance from breeding sites increases, and is especially apparent for arboreal species. Recently however, the use of artificial arboreal refugia has been successful in capturing a variety of species of arboreal anurans (Boughton et al. 2000; Moulton et al. 1996). Through monitoring of artificial arboreal refugia that mimic natural treefrog diurnal habitat, we can monitor behavior at great distances from breeding sites without affecting movement activities, and elucidate the biotic and abiotic factors that contribute to an individual's choice of microhabitat on a daily and seasonal basis.

The objectives of our study were to determine how environmental factors affect microhabitat use by the gray treefrog (*Hyla versicolor*), and to describe previously unreported aspects of their natural history away from breeding sites that may help to determine the importance of terrestrial habitat features and the quality of habitat patches. We used both mark-release/recapture techniques and radiotracking to: 1) test the effects of weather variables on frequency of captures within artificial refugia, and on daily microhabitat locations of individuals implanted with radiotransmitters, 2) test the effect of tree characteristics, such as species and diameter, on use of refugia, and 3) report on the extent of non-breeding season foraging grounds, and the microhabitat in which treefrogs overwinter. These aspects of treefrog natural history and terrestrial habitat use provide important data regarding the biological factors that contribute to overall patch quality for amphibians.

METHODS

We captured gray treefrogs at the Baskett Wildlife Research Area in southern Boone County, Missouri in artificial arboreal refugia (Appendix A). Artificial refugia consist of 60 cm long sections of 3.8 cm diameter black plastic pipe. Refugia are sealed at the bottom, and designed to retain rainwater at a depth of 15 cm. The upper opening is unobstructed, and frogs are not restrained within refugia. Refugia were placed on large diameter deciduous trees and arranged into seven paired transects (i.e., two nearby pipes at each distance) extending from breeding sites into secondary growth oak/hickory (*Quercus spp.*/*Carya spp.*) forest with sugar maple (*Acer saccharum*) understory. Paired artificial refugia were located at intervals of 1, 5, 15, 35, 60, 100, and 200 m, for a total of 98 refugia. We monitored refugia for the presence of treefrogs every other day during daylight hours, for the duration of treefrog activity periods (typically April through November) from the fall of 2002 to the spring of 2005. If present, frogs were extracted with a sponge for processing (Boughton et al. 2000). We measured snout-vent length with a plastic ruler for all individuals at least once per season, and body mass with a Pesola spring scale starting in the spring of 2004. If not previously marked, we excised toes to generate a unique toe-clip identification. After processing, frogs were returned to the pipes and placed back on the tree. Additionally, we performed nocturnal monitoring of the three aquatic breeding sites to illustrate that individuals captured in artificial refugia were members of the local breeding population.

A subset of large (>8.5g) individuals (*N*=22) captured within pipe-traps were implanted with 0.85 g radiotransmitters from Holohil Industries and relocated on a daily basis to increase the resolution of data regarding habitat use. Individuals were implanted with transmitters within 24 hrs of capture, and released after a recovery period of approximately 24 hrs (Appendix B). Daily relocations included a description of microhabitat location (i.e., approximate height in tree, under leaf litter, etc.), and plotting of the location in UTM coordinates.

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 the effect of ambient weather on captures of gray treefrogs. The relative contribution of weather conditions on artificial arboreal refugia use was assessed using simple linear regression and Pearson correlation analyses. Weather variables to be used in regression comparisons were selected by significant correlations with artificial refugia captures or microhabitat use of radiotracked individuals. Multiple weather variables that were significantly correlated with each other ($P<0.05$) were omitted from analyses, and only the variable most highly correlated with the data were included in regression models. Variables tested include temperature (minimum, maximum, mean), humidity (minimum, maximum), windspeed (5 second maximum, 2 minute maximum, mean) and total rainfall (day of capture, day of capture-1, day of capture-2, day of capture-3, and day of capture-4).

To determine the potential benefit of desiccation reduction for individuals in arboreal refugia, plastic mesh (0.5 cm) was formed into the shape and size of an artificial refuge to be used for dehydration trials. Four replicate pairs of mesh refugia and solid artificial refugia were placed side-by-side on trees within the confines of the arrays used during the mark-recapture portion of this study. Individuals used in the desiccation trials were collected from a nearby pond (outside of the experimental arrays) and haphazardly chosen to remain either in a mesh pipe or in a solid artificial refuge that was capped with mesh to exclude resident treefrogs, and prevent escape. We weighed individuals (to the nearest 0.1 g) with a hand-held Pesola spring-scale before dehydration trials and at two additional six-hour intervals (0000, 0600, & 1200 CST). Replicate trials were performed on four non-consecutive nights from 28 June to 9 July 2004. Upon conclusion of the dessication trials, we returned all individuals to the breeding pond from which they were collected. We compared the average body mass of individuals before and after the dessication trials with a two-tailed T-test.

We measured the diameter at breast height (DBH) of trees in which pipes were placed, with a DBH tape to the nearest 0.1 cm, to determine if observed capture frequencies differed among trees. The effect of tree diameter was analyzed by linear regression analysis. Additionally, trees were identified to species and grouped into categories (e.g., oaks, hickories, etc.) for tests of goodness-of-fit (G-tests). Expected treefrog capture frequencies for each tree category were based on the relative proportion of each tree type containing artificial refugia.

RESULTS

A total of 109 adult treefrogs (42 female and 67 male) were captured 595 times in artificial arboreal refugia. We observed 109 movements between pipes by 36 individuals, with the majority (61%) of movements occurring among paired artificial retreats at each distance within transects. Twenty of the 22 individuals implanted with radiotransmitters were tracked for an average of 24 (±0.9 SE) days during the fall post-breeding periods of 2003 and 2004 (*N*=485 relocations), and followed to their overwintering microhabitat. Two individuals were omitted from analyses due to low numbers of relocation points. Ninety-five percent kernal space-use estimates varied from 5.6 m^2 to 2747.8 m^2 and averaged 530.2 m^2 (±186.2 SE; Fig. 1). Radiotracked individuals moved an average of 8.30 times (±0.96 SE) with an average of 6.11 m (±0.71 SE) per move. The average total linear distance moved within foraging grounds was 52.83 m (±8.61 SE), and the longest single movement recorded was 80.31 m. Many individuals made multiple short distance foraging movements while temperatures remained above freezing, and a single longer distance movement to reach overwintering microhabitat (e.g., Fig. 1C).

Eighteen individuals were ultimately tracked to the ground for overwintering (Fig. 2). The remaining two individuals were located in trees as batteries failed, and one individual was extracted from a small opening in the distal end of a horizontal white oak (*Quercus alba*) branch approximately 9.2 m off the ground, while the other was located at ~15 m and could not be reached. 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 (Fig. 2), presumably following heavy rains. Although, a few individuals (N=3) utilized existing underground burrows.

Abiotic factors

Minimum humidity, previous 3 days cumulative rainfall, and maximum temperature were the best predictors of the number of treefrog captures. Humidity ($F_{1,107}$=4.487, P=0.037) and rainfall ($F_{1,107}$=8.745, P=0.004) were negatively correlated with pipe captures, and temperature ($F_{1,107}$=3.536, P=0.063) was positively associated with captures (Fig. 3). The relative height of tracked animals also varied in accordance with the environmental variables that best predicted captures (Fig. 4). Furthermore, cool days with low humidity resulted in high proportions of individuals within leaf litter, and hotter, dryer days increased use of artificial arboreal retreat sites (Table 1). High 3-day rainfall accumulation values increased frog use of the tree canopy, while calendar date was the best predictor of soil microhabitat use (Table 1). The weight of frogs held captive in screened pipe-traps decreased from 6.49 g to 4.61 g ($T_{2\text{-tail}}$=6.063, *df*=19, *P*=0.004; Fig. 5) over the 12 hr testing period, whereas the frogs contained within ‘normal’ pipe-traps experienced a slight non-significant gain in mass from 6.36 g to 6.45 g ($T_{2\text{-tail}}$=-0.250, *df*=19, *P*=0.815; Fig. 5).

Biotic factors

Thirty-two trees with artificial refugia never recorded a treefrog capture. Considering all trees with refugia attached, we observed an increase in total treefrog captures as tree diameter increased (Fig. 6). Also, there was an effect of tree species on total captures (*G*=18.26, *df*=5, *P*=0.05; Table 2), with red oaks being underutilized, and white oaks supporting a greater number of captures than expected (*G*=13.36, *df*=1, *P*< 0.001). Further, there was an effect of tree species on relative captures of males and females, with males using more red oaks than predicted and females using more white oaks than predicted. There was no difference in total captures among dead trees and live trees of all species combined (*G*=0.47, *df*=1, *P*=0.49, Table 2). Similarly, we detected no effects of tree species or diameter on treefrog captures, whether living or dead, when considering only unique captures (i.e., the number of different individuals captured) per tree.

The average mass of individuals captured in artificial arboreal refugia was greater than the average mass of individuals captured by hand at breeding ponds ($T_{2\text{-tail}}$=7.550, *df*=146, *P*<0.001; Fig. 7). We found no difference in mass among females captured in refugia and those captured at ponds ($T_{2\text{-tail}}$=0.729, *df*=87, *P*=0.468), and conversely, the mass of males increased significantly among individuals captured in refugia compared to those captured at ponds ($T_{2\text{-tail}}=3.278$, $df=745$, $P=0.001$; Fig. 7).

DISCUSSION

Breeding ponds are obviously important to amphibians that have indirect development (i.e., an aquatic larval stage), but population dynamics are not solely dependent on the quality of the aquatic breeding habitat. Therefore, in a metapopulation context, habitat patches containing pond-breeding amphibian populations should not be characterized by aquatic habitat alone (Marsh and Trenham 2001). It is increasingly clear that the terrestrial habitat adjacent to breeding sites is important for many non-breeding activities (Semlitsch 1998, 2000) such as migration, dispersal, foraging, and overwintering. What remains to be identified are the critical features of the terrestrial landscape that are utilized for life-history activities, the environmental variables that drive the microhabitat choices of individuals, and the evolutionary fitness consequences of their choices.

Gray treefrogs breed over the course of several months in Missouri (Johnson 2000). During the breeding season, males actively call at ponds for several consecutive nights before leaving the chorus, presumably to forage and subsequently return (Chapter 2A). Females however, are present at breeding ponds only long enough to mate and deposit eggs, once or twice per breeding season (Ritke et al. 1990; Chapter 2A). The habits of gray treefrogs are largely unknown when absent from breeding sites during either the breeding or non-breeding seasons, however they are generally believed to require some amount of forested habitat adjacent to breeding sites in which to take up residence in treeholes diurnally. Furthermore, anecdotal reports have indicated that treefrogs may overwinter in treeholes or on the ground (Burkholder 1998), and that treeholes are extensively used as foraging locations (Park et al. 1950). In our study gray treefrogs utilized artificial arboreal retreat sites as diurnal refugia during both the breeding and non-breeding seasons from April through November.

We found relatively small space-use estimates and frequent movements only among pipes separated by less than 10 m. Few estimates of space-use overlapped among the two years of tracking, and none within years. Additionally, overwintering locations were in close proximity to inferred arboreal foraging grounds. Other radiotracking studies on pond-breeding amphibians have generated 95% kernal space-use estimates of 75-1842 m^2 (Birchfield 2002; *Rana clamitans*) and 104-4609 m^2 (Rittenhouse 2002; *Ambystoma maculatum*), which overlap those estimated in our study, but do not encompass the lower limit of our space-use estimates. However, these previous radiotracking studies were performed while individuals were migrating from breeding sites, while our estimates solely encompass post-breeding foraging activities.

For most amphibians, microhabitat choice in a very important decision due to their physiology. As ectotherms, ambient temperature plays a critical role in determining activity levels (Wygoda 1989), as does evaporative water loss (Wygoda 1984), given the permeability of their skin. We monitored microhabitat use by the presence or absence of individuals in pipe-traps, and by the specific locations of individuals implanted with radiotransmitters. Environmental variables were significantly correlated with the use of different categories of microhabitat by animals implanted with radiotransmitters. Early in the fall, when conditions were favorable (i.e., high humidity and rainfall) frogs moved upwards in the canopy, which could suggest that treetops may be the preferred microhabitat for foraging or escaping predation, or both. Unfavorable conditions (i.e., high maximum daily temperatures or low minimum relative humidity) resulted in increased use of artificial retreats, presumably to take advantage of the standing water within (Appendix A), and decrease evaporative water loss. As the weather cooled, animals moved towards the ground in preparation for movement to hibernation sites in the soil beneath leaf litter. It is unknown if foraging behavior continued once on the ground, although it is unlikely considering that temperatures were approaching 0°C.

Our data provide two lines of evidence that diurnal use of refugia by treefrogs serves to protect them from exposure to harsh environmental conditions. First, we found that individuals enclosed within ‘solid’ artificial refugia maintained hydration, while those held in screened refugia quickly desiccated. The rapid rate of evaporative water loss is surprising for a treefrog, given previous comparisons among terrestrial and arboreal anurans (Wygoda 1984). Second, the relationship between increased number of captures and decreasing relative humidity or rainfall (or increasing maximum temperature) supports the suggestion of Buchanan (1988) that diurnal refugia are important for maintaining proper physiological functions of temperate treefrogs.

Furthermore, Mahan and Johnson (Appendix C) 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 indicated that retreats were utilized in-between nocturnal foraging bouts. Based on the types of insects collected from the stomach samples analyzed by Mahan and Johnson (Appendix C), it appears that foraging occurs arboreally, and thus the use of diurnal retreats appears to be twofold: to provide escape from the elements and to provide quick access to foraging sites. The abundance of treeholes could therefore be a critical component determining the extent of terrestrial habitat used by the gray treefrog. Additionally, Johnson (Appendix A) found a low percentage of multiple frogs occupying the same artificial retreat, and Buchanan (1988) found some evidence of territoriality in other *Hyla*. If territoriality influences natural refugia use, the carrying capacity of the local populations could be reflected by refugia abundances.

Treefrogs do not appear to choose their refugia haphazardly, as we found significant differences in total captures among tree species. We found an increased number of captures in pipes on white oak trees than expected, and a concomitant reduction in the use of red oak trees. The bark of white oaks and red oaks differ, with white oaks having much softer, convoluted bark than red oaks (pers. obs.). Differences in bark composition may affect insect abundances, and could have an effect on the formation of treeholes among tree species. Whether the insect community differs in composition or abundance among the oaks in this study is unknown, but increased use (i.e., total number of captures) of artificial diurnal refugia seems likely to be the result of enhanced foraging site quality that can be exploited over the course of multiple foraging bouts. If insect abundances are uniform, variation in the number or quality of refugia may be a more critical determinant of tree species use than quality of foraging resources. Frogs did appear to occur more frequently in larger diameter trees, however we have no clear prediction regarding use of pipes based on tree diameter. For example, large diameter trees are most likely older, and support a larger number of natural treeholes. Therefore, we may expect fewer captures in our artificial pipes placed in large trees simply because suitable alternative to artificial refugia already exist in those trees. Elucidation of this relationship is not improved by our observed lack of differences regarding the use of artificial refugia in dead trees (i.e., snags) versus live trees, as dead trees almost certainly contain a greater number of natural arboreal refugia. Or perhaps we should expect more captures tree diameter increases, because more individuals would identify large trees as appropriate sites in which to search for natural refugia. In either case, we found no effect of tree species or diameter on the number of unique individuals captured in artificial refugia, which further supports the contention that spacing of individuals could be important in gray treefrog habitat use.

While delineation of core habitat required by a population is certainly important, and for critically imperiled species should probably be determined on a site-by-site basis, generalizations of appropriate habitat protection requirements for pond-breeding amphibians that ignore the quality of the habitat can be deceiving. Gray treefrogs require terrestrial habitat adjacent to breeding sites that contains mature trees in which to forage, an abundance of treeholes to prevent desiccation. An adequate leaf litter layer is also necessary to protect overwintering treefrogs from cold winter temperatures. Our observations suggest that aspects of patch quality could be affected by fragmentation and habitat loss. Reduction in the number of trees available within patches could reduce the carrying capacity of local populations, especially if territorial behavior restricts use of treeholes to single individuals. Edge effects such as increased temperature and decreased humidity along forest/non-forest boundaries (Murcia 1995) may impact foraging behavior of gray treefrogs and limit the efficiency with which they can obtain resources. The effects of decreased foraging efficiency could manifest as reduction in female fecundity per season (Ritke et al. 1990) and further diminish population sizes. From a regional amphibian decline perspective, reductions in local population sizes diminish the number of dispersers available to perform inter-patch movements and increase local extinction probability.

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Table 1. Comparison of the effect of tree species on treefrog captures.

	Unique Captures			**All Captures**		
Tree Category	**Observed Frequency**	**Expected Frequency**	***P***	**Observed Frequency**	**Expected Frequency**	***P***
Ash	10	9.25	0.99	38	38.89	0.05
Elm	12	9.25		48	38.89	
Hickory	14	11.56		59	48.61	
Red Oak	32	36.98		118	155.54	
Maple	40	43.92		180	184.70	
White Oak	33	30.05		150	126.38	
Living	15	12.26	0.43	44	48.56	0.49
Dead	164	166.74		665	660.44	

Table 2. Correlation (Pearson's *R*) of weather variables with proportion of individuals in each microhabitat type during radiotracking.

	Leaves	**Soil**	**Tree**	**Pipe**
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

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

Figure 1. Estimated space-use estimates for 5 individuals illustrating: small foraging habitat area (**A**), linear movements (**B**), disjunct foraging areas (**C, D, & E**), overwintering sites distant from foraging areas (**C & D**), and overwintering sites in proximity to high-use foraging locations (**A, B, E**). Stars represent overwintering site locations, squares indicate artificial arboreal refugia locations, and circles denote individual location points. Animal identification number and sex are listed in each panel.

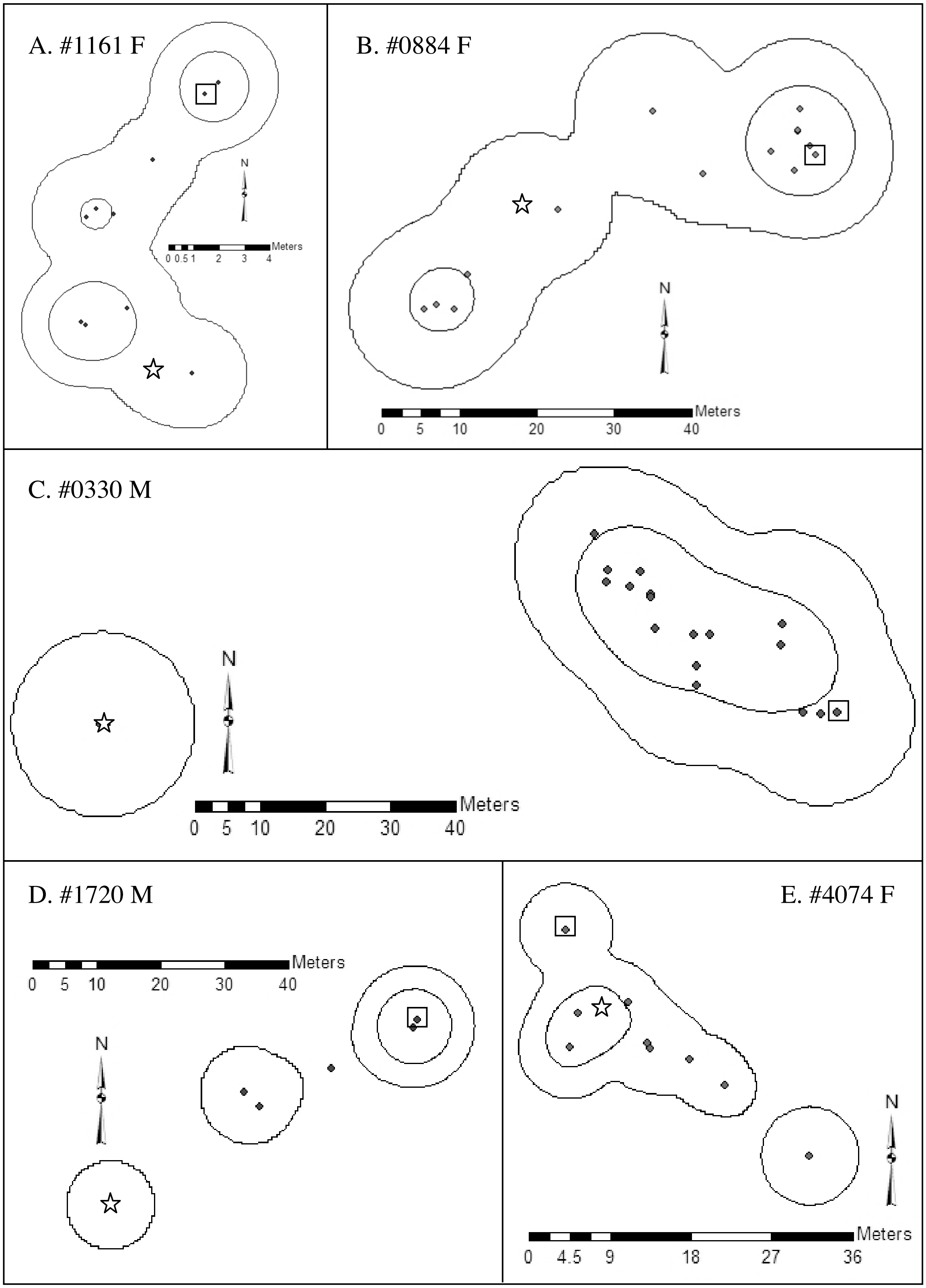

A. #1161 F
N
Meters
0 0.5 1 2 3 4
B. #0884 F
N
Meters
0 5 10 20 30 40
C. #0330 M
N
Meters
0 5 10 20 30 40
D. #1720 M
Meters
0 5 10 20 30 40
N
E. #4074 F
N
Meters
0 4.5 9 18 27 36

Figure 2. Examples of two overwintering gray treefrog microhabitat locations.

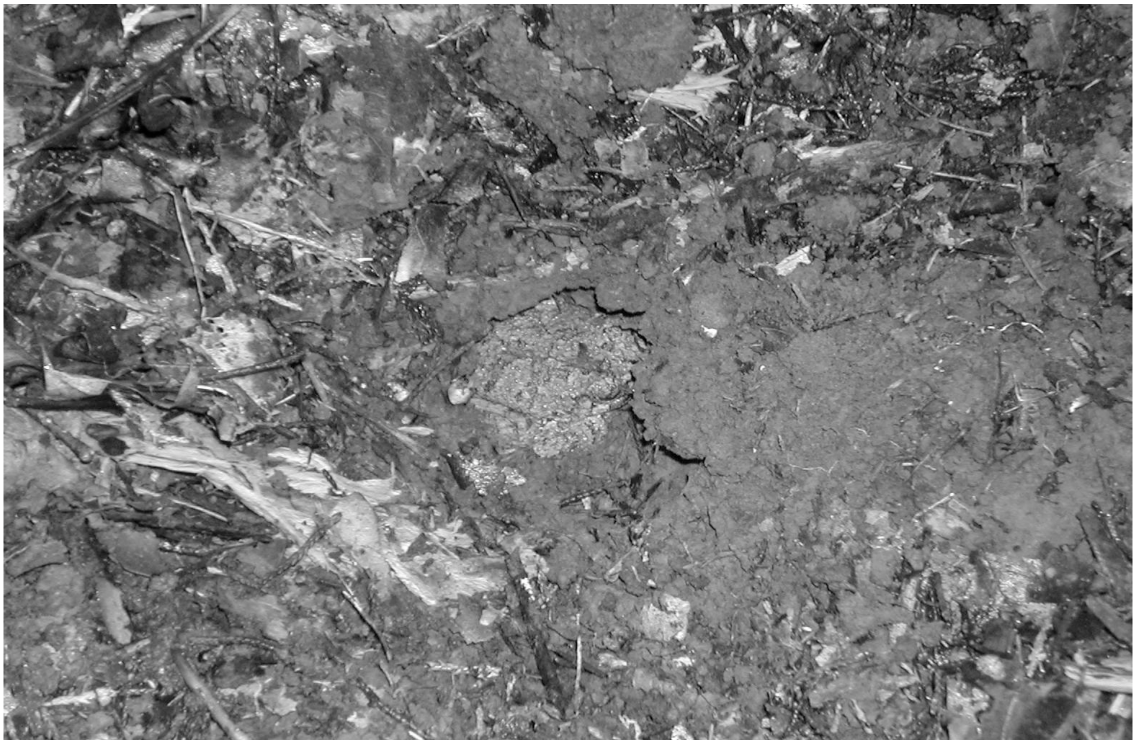


Figure 3. Scatter plots of three weather variables most highly correlated with treefrog captures (natural log transformed). **A)** Maximum temperature, **B)** minimum humidity, and **C)** accumulated three-day rainfall.

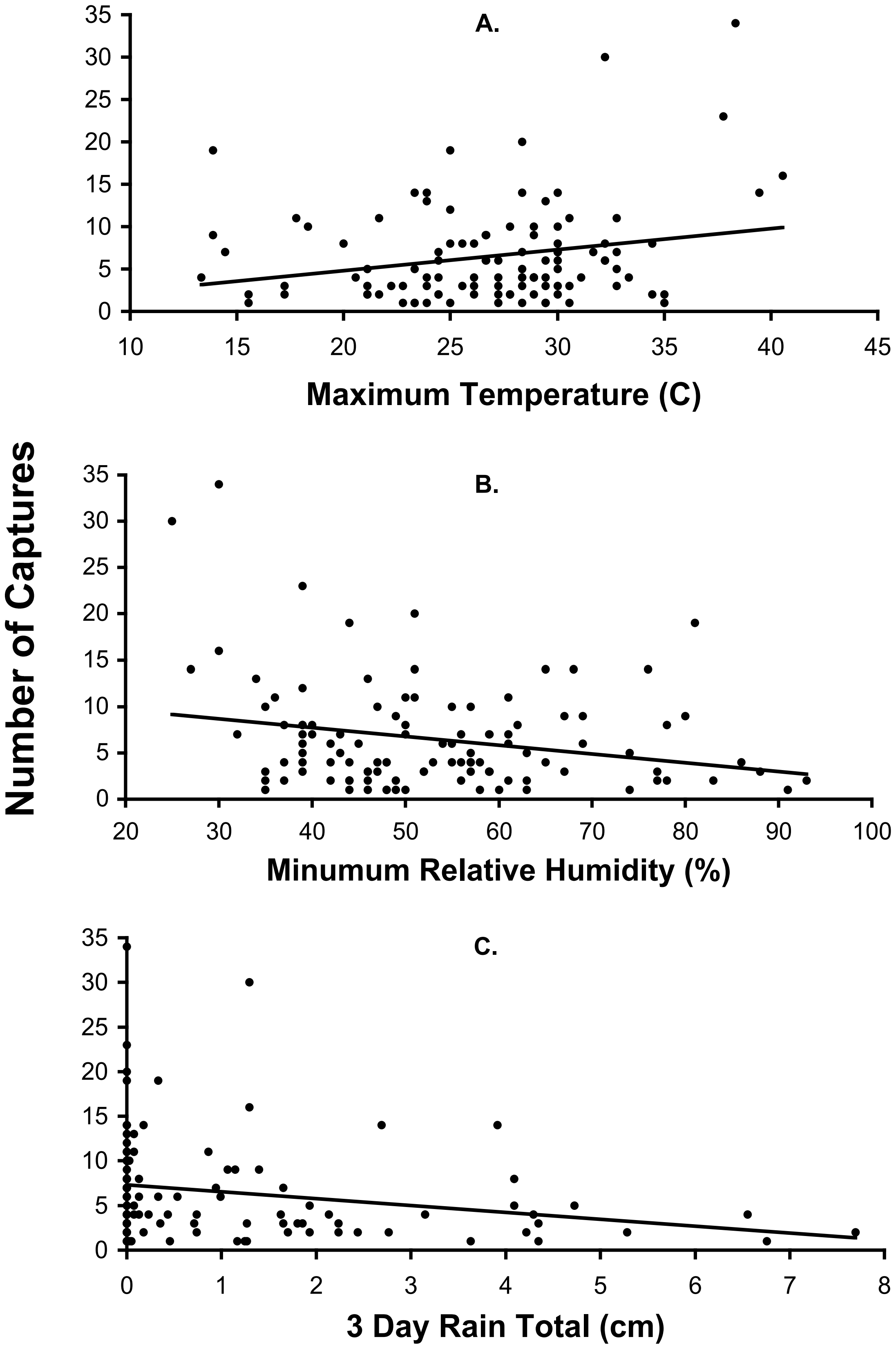
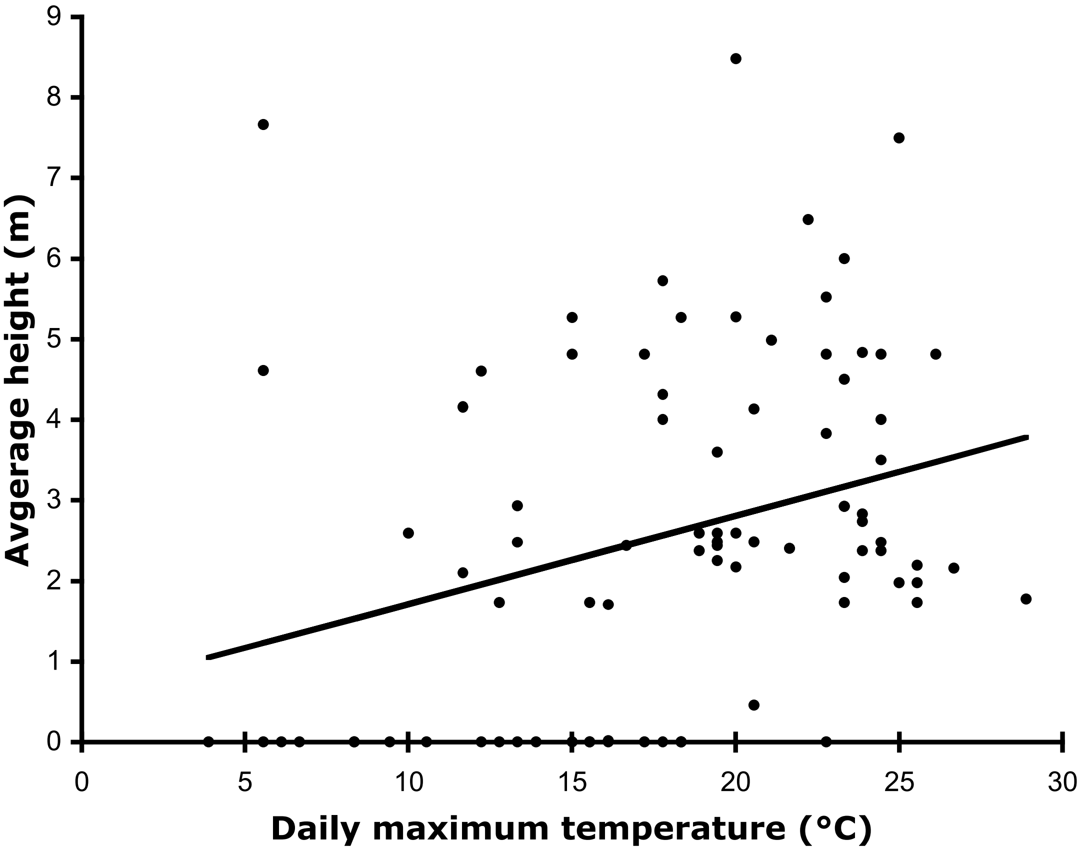

Number of Captures
A.
35
30
25
20
15
10
5
0
10
15
20
25
30
35
40
45
Maximum Temperature (C)
B.
35
30
25
20
15
10
5
0
20
30
40
50
60
70
80
90
100
Minumum Relative Humidity (%)
C.
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
3 Day Rain Total (cm)

Figure 4. Effect of temperature on height of implanted frogs.



Avgerage height (m)
0
1
2
3
4
5
6
7
8
9
0
5
10
15
20
25
30
Daily maximum temperature (°C)

Figure 5. Average weight (±SE) among individuals within ‘normal’ artificial arboreal refugia and screened pipes.

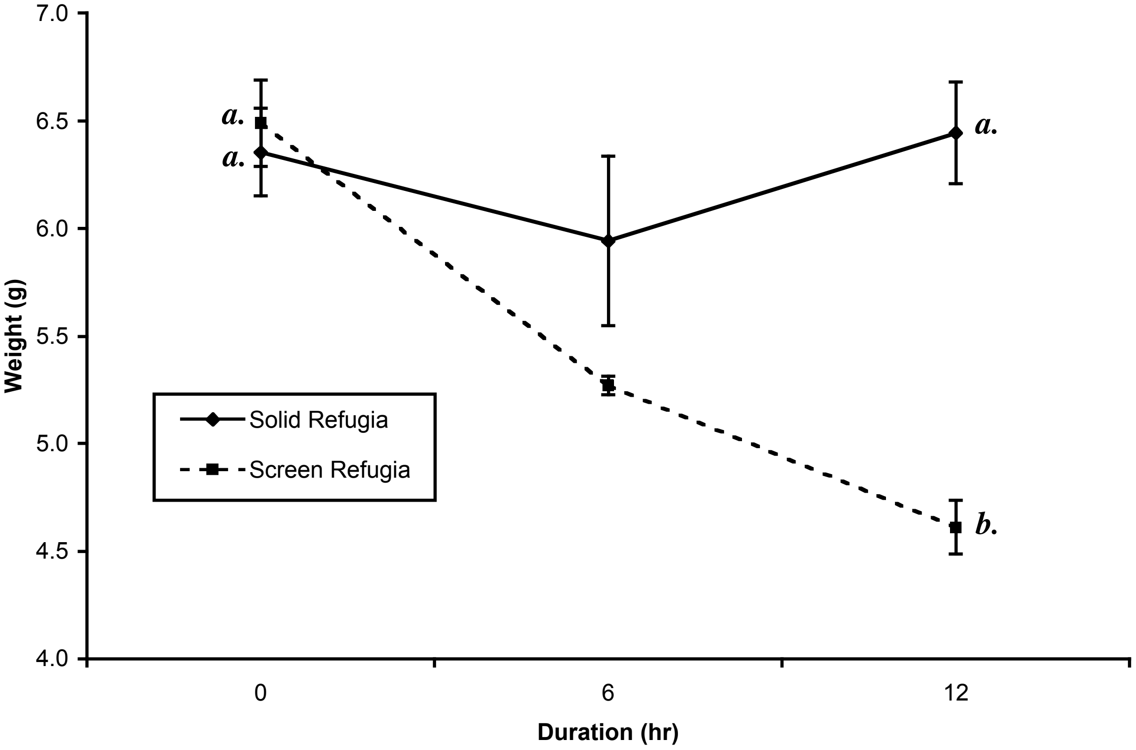
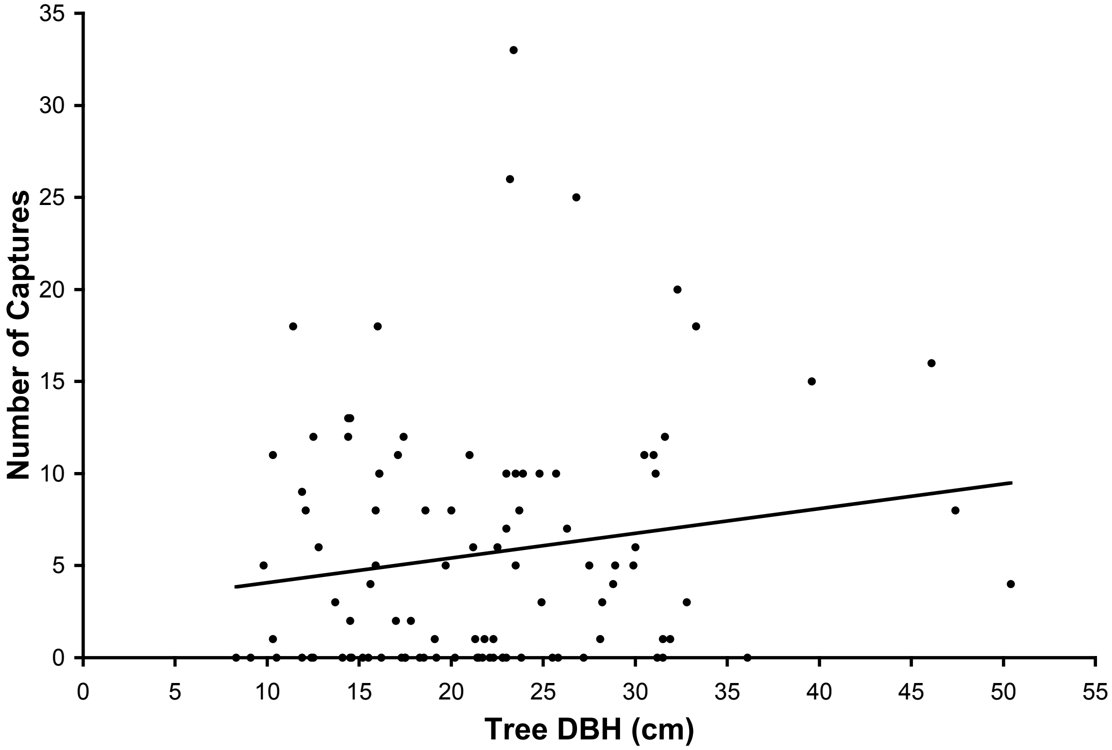
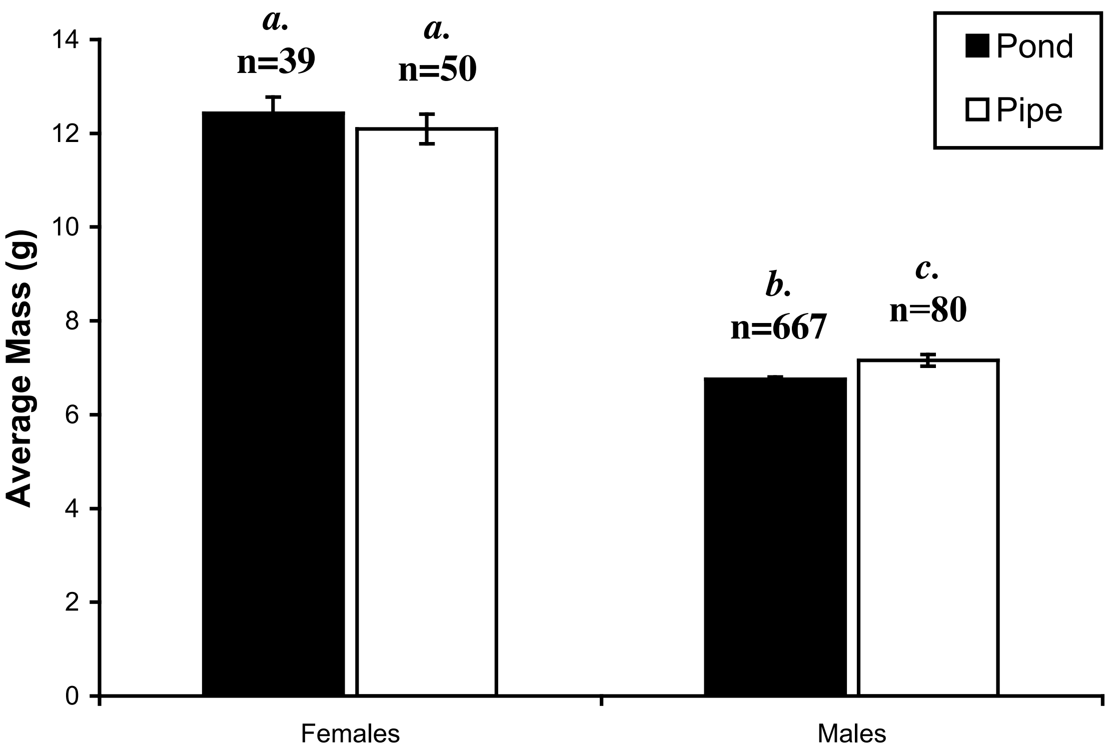

7.0
6.5
6.0
5.5
5.0
4.5
4.0
Weight (g)
0
6
12
Duration (hr)
a.
a.
a.
b.
Solid Refugia
Screen Refugia

Figure 6. Effect of tree diameter on treefrog captures in artificial arboreal retreats



Number of Captures
0
5
10
15
20
25
30
35
Tree DBH (cm)
0
5
10
15
20
25
30
35
40
45
50
55

Figure 7. Comparison of adult treefrogs weighed at breeding sites versus arboreal refugia (±SE).



14
12
10
8
6
4
2
0
Average Mass (g)
a.
n=39
a.
n=50
b.
n=667
c.
n=80
Females
Males
■ Pond
☐ Pipe

Chapter 3

EFFECT OF INTER-POND DISTANCE AND MATRIX HABITAT ON MOVEMENTS OF THE GRAY TREEFROG, *HYLA VERSICOLOR*, IN EXPERIMENTAL POND ARRAYS

Jarrett R. Johnson and Raymond D. Semlitsch

ABSTRACT

The need to understand the effects of habitat fragmentation and preserve naturally functioning populations within remaining habitat patches has led to an increase in the use of metapopulation theory for a variety of taxa. From an ecological and conservation perspective, an understanding of the effects of inter-pond distance and habitat matrix are crucial to implementing programs aimed at protecting natural population dynamics. Pond-breeding amphibians represent an appropriate group in which to study metapopulations because the local habitat patch is centered on a breeding pond (or ponds) that often can be identified and measured. Many pond-breeding amphibian species are forest-associated, and habitat destruction may inhibit dispersal among local populations and lead to increased risk of local extinctions. To determine how amphibian dispersal between breeding sites is limited by distance and matrix habitat, we monitored movement rates of the gray treefrog (*Hyla versicolor*) among experimental pools separated by varying distances and habitat types. Inter-pond movements were recorded using a combination of mark-release/recapture techniques and DNA fingerprinting using microsatellite markers. Movements through old-field matrix were significantly less frequent than those through oak/hickory forest for juveniles, and the distance between ponds significantly influenced the frequency of adult movements within and among consecutive breeding seasons. Further, habitat and distance interact to produce an overall negative effect on longer movements through old-field habitat. In fact, ponds separated by 180 m experienced almost a 75% decrease in inter-pond movements. These results indicate that gray treefrogs are capable of moving large distances through terrestrial habitat, and that old-field habitat poses a greater resistance to movements than inter-pond distance. Furthermore, habitat composition and inter-pond distance appear to have varying influences on movements depending on life history stage. We suggest that interruption of continuous forested habitat will decrease the frequency of dispersal among local populations of forest-associated amphibians and could subsequently alter natural population dynamics and thus regional persistence of pond-breeding amphibians.

INTRODUCTION

It has long been recognized that populations of most organisms are naturally patchily distributed at some scale (e.g., Wright 1940; Andrewartha and Birch 1954). The structure and dynamics of spatially clumped populations vary according to the distribution of resource patches and the degree of individual movements among patches. The capacity for inter-patch movements differs among taxa due to morphology and behavior, and is affected by many factors including patch size, patch quality, inter-patch distance, and regional habitat composition. Patchy populations exist when dispersal movements among patches are frequent and sufficient enough to limit local extinctions (Harrison 1991). Furthermore, if habitat patches are indistinct or temporally variable, such that habitat beyond patch 'boundaries' is not completely unsuitable for population persistence, patchy population dynamics prevail. Conversely, metapopulations in the classical sense are composed of subpopulations within definable habitat patches, separated by expanses of inhospitable matrix, with frequent local extinctions and recolonization of extinct patches that does not occur immediately (Levins 1969, 1970). For metapopulations, regional/landscape dynamics are more important than the local dynamics of subpopulations (i.e., patches). Systems of isolated populations (i.e., non-equilibrium metapopulations) rarely (if ever) exchange individuals, and experience population dynamics dominated by factors within the local patch, and have no potential for recolonization of extinct sites (Harrison 1991).

The processes that define a group of interacting populations as a metapopulation or a patchily distributed population lie along a continuum, therefore from an ecological and conservation perspective, it is important to understand the relative importance of both regional and local dynamics, when considering the prospects of species persistence in a landscape. The importance of determining which factors are driving patch occupancy rates increases as landscapes are modified by habitat destruction and fragmentation.

Habitat destruction and alteration has been implicated in concern regarding worldwide amphibian declines, and recent evidence suggests that amphibians are declining more rapidly than other taxa (Stuart et al. 2004) in response to environmental changes. Therefore, efforts directed towards understanding the effects of local extinctions of amphibian populations are crucial. For temperate pond-breeding amphibians, the application of metapopulation concepts has been particularly appealing (e.g., Gill 1978; Berven and Grudzien 1990; Sjögren 1991; Sjögren-Gulve 1994; Sinsch 1992; Driscoll 1997), but have often been based on the presumptions that aquatic breeding sites comprise discrete patch locations and amphibians have low dispersal capability. However, recent reviews have suggested that traditional metapopulation models cannot be generally applied to all pond-breeding amphibians because of issues regarding the delineation of patch boundaries (Marsh and Trenham 2001) and underestimates of dispersal movements (Smith and Green 2005). Yet, descriptions of core terrestrial habitat for local populations surrounding wetlands support the delineation of pond-based patches (Semlitsch 1998, 2000; Semlitsch and Jensen 2001; Semlitsch and Bodie 2003).

Whether natural populations are best described a metapopulations or patchy populations, depends on the frequency of dispersal among sites (Harrison 1991). In theory, destruction of natural habitat should decrease the probability of dispersal between remnant habitat fragments by effectively increasing isolation among patches with both decreases in the number of suitable sites and increases in the extent of matrix (Taylor et al. 1993; Fahrig and Merriam 1994). Reduction in the magnitude of dispersers between sites may force naturally patchy populations into discrete localized units that are ultimately forced to become metapopulations. Therefore, the application of metapopulation concepts to the fields of conservation and ecology are becoming increasingly appropriate to a wide variety of taxa regardless of the dynamics of naturally structured populations.

However, investigations regarding the effect of fragmentation on population dynamics have focused primarily on patch isolation in terms of distance, and have ignored the effect of matrix composition (Rickets 2001; Vandermeer and Carvajal 2001). Observed landscape-level distributions of pond-breeding amphibians from previous metapopulation studies have lead to the conclusion that inter-pond distance (i.e., isolation) is a major factor in determining the probability of pond occupancy (Laan and Verboom 1990; Reading et al. 1991; Sjögren 1991; Sjögren-Gulve 1994; Halley et al. 1996; Moilanen and Hanski 1998). Other studies have found that isolation was unimportant to occupancy (Driscoll 1997; Marsh et al. 1999; Drechsler and Wissel 1998). An additional factor that could be contributing to observed pond occupancy rates is the composition of matrix habitat, and combined with distance may provide more detailed information regarding the realized dynamics of pond-breeding amphibian populations (Laan and Verboom 1990; Fahrig et al. 1995; Kareiva and Wennergren 1995; Lehtinen et al. 1999; Joly et al. 2001). The composition of terrestrial habitat through which dispersers must travel likely has impacts on amphibian population persistence and site occupancy patterns (Reh and Seitz 1990; Denton and Beebee 1993; Fahrig et al. 1995; Moilanen and Hanski 1998; Lehtinen et al. 1999; Marsh et al. 1999; Pope et al. 2000), however, experimental assessments of movement capabilities through alternative habitat types for any taxa are rare (but see Pither and Taylor 1998; Marsh et al. 1999, 2004).

In response to calls for data regarding dispersal behavior and the effects of matrix habitat on interactions among local populations within a landscape, we tested the frequency of movements by a forest-associated species, the gray treefrog (*Hyla versicolor*), among artificial ponds experimentally arranged within fragmented deciduous forest habitat. Forest or field habitat separated ponds at two different spatial scales and movements were detected using both genetic markers and mark-release/recapture techniques. We predicted that movements through old-field habitat would be fewer relative to movements through forest, and that short movements would outnumber long movements. Furthermore, we expected that distance and habitat type would interact to result in the fewest number of movements occurring in long-distance old-field treatments. In addition, we provide data regarding adult breeding pond philopatry and juvenile dispersal tendency as they pertain to the observed population dynamics.

METHODS

Experimental pond arrays

This study was conducted at the Thomas Baskett Wildlife Research Area (BWRA) in Boone County Missouri, USA. Three replicate locations were selected in which to place cattle tank ponds (2.4 m diameter) in arrays that provided within-site replication of two inter-pond distances and two habitat treatments. Ponds were dug into the ground within forested habitat at existing forest-field edges with a backhoe and back-filled by hand until the pond edges were flush with the ground. Each array consisted of a single central pond surrounded by either six (sites 1 & 2) or five (site 3) peripheral ponds 60 or 180 m distant to the center through either forest or field habitat (Fig. 1). The scale of the experimental design corresponds with typical movement distances within breeding seasons (60 m; Chapter 1), and non-breeding season migratory movements to overwintering sites (180 m; Chapter 2A). Our results therefore are representative of movements appropriate for the dynamics of local treefrog populations on a temporal scale of a single year. We were not attempting to elucidate maximum dispersal distances of gray treefrogs, but rather to determine the effect of habitat on frequency of movements and thus population dynamics. When combined with information regarding typical dispersal distances, these data can be used to determine the wetland density appropriate for maintenance of connections through habitat of varying quality in fragmented landscapes.

Cattle tanks were filled by rainwater and by hose from a truck with a water tank until the water level was within 4-5 cm from the pond edge. Small sections of plastic screening were attached to the adjacent soil and allowed to hang into the water to provide a means of escape for animals (amphibian or otherwise) attempting to leave the pond. Each pond received 2 kg of deciduous leaf litter each spring at which time any old debris was removed. Zooplankton communities were established via inoculation with a 1 L concentrated zooplankton and periphyton aliquots collected from nearby natural ponds.

Location and release of unique individuals

During the summer of 2000 we collected gray treefrog tadpoles from numerous natural ponds (*N*=20) throughout central Missouri, including many ponds (*N*=10) from within BWRA. We extracted DNA from 15 to 20 tadpoles from each site using Qiagen DNEasy tissue extraction kits and amplified microsatellite loci on an MJ Research thermal cycler.

Individuals were screened at many loci for the presence of unique alleles found at sites outside the boundary of BWRA but absent from individuals naturally occurring within the study site. Scoring of alleles was performed at the University of Missouri DNA Core Facility on an ABI 227 sequencer and analyzed using ABI Prism Genotyper software. One site near Overton Missouri (OV) contained the highest proportion of individuals with alleles unique from BWRA at two loci (19F9G, and 6 m4E; Krenz et al. 1999) and was selected as the source of individuals to be translocated for this study.

During the springs of 2002 and 2003 mating pairs of gray treefrogs were collected from the OV site and allowed to deposit their eggs in individual plastic containers containing a small amount of charcoal-filtered and UV-irradiated water in the laboratory. Toes from parent frogs were excised for tissue to be used in genotyping and to provide a unique toe-clip identification. Eggs hatched within a few days and were divided into three containers (per family) containing 50% filtered and irradiated water and 50% pond water from the cattle tank in which they were to be released. During this time, DNA was extracted from excised toes, amplified via PCR, and genotyped. Clutches generated by parents that were not homozygous for at least one unique allele were returned to the OV site as well as all adult frogs captured. Clutches that contained either or both unique alleles from the two loci were transferred to the central pond in each of the three arrays following absorption of yolk sacs. To reduce mortality from predation by salamander (*Ambystoma*) or odonate (*Anax*) larvae, tadpoles were released into screened cages placed within the pond and allowed to grow beyond the gape limitations of the predators (several weeks) before being allowed to move throughout the pond.

Mark-release/recapture techniques

High levels of colonization of cattle tank ponds from natural ponds at BWRA provided an additional opportunity to monitor the movements of individuals throughout each of the replicate cattle tank arrays. Late-stage tadpoles (front and back limbs present) from each cattle tank were removed and placed into water-filled containers housed within completely enclosed screened pens and allowed to complete metamorphosis. We measured newly metamorphosed froglets (<3 d), excised toes to generate unique marks, and released them into the leaf litter adjacent to the cattle tank edge. Adult treefrogs were also given unique toe-clips during regular nighttime visits to small breeding choruses (usually <10 calling males) at each cattle tank pond. We divided mark-release/recapture data for adults into movements that occurred during a single breeding season and events occurring among breeding seasons.

Analyses

To determine the interaction of inter-pond distance and matrix habitat type, all movement data were analyzed using 2-factor log-likelihood-ratio Goodness of Fit Tests (G_{Int}). We performed analyses on: all movements combined, juvenile movements only, adult movements only, all among-season movements, juvenile among-season movements, adult among-season movements, adult within-season movements, and genetic data only. We also calculated single factor effects of distance (G_{Dis}) and habitat type (G_{Hab}) on all above categories using G-tests. We do not report the results of tests with *P*-values >0.1.

RESULTS

Dispersal of unique genotypes

A total of 11 treefrog pairs were captured at the OV site in 2002 and 2003. Ten of the 11 pairs deposited eggs, five resulting from parents with genotypes that contained the alleles unique to the OV site and were used to stock ponds in this study. A total of 629 tadpoles were released in the central cattle tank of each of the three experimental arrays in 2002, and 725 tadpoles were released in each central cattle tank in 2003. Of these 1,354 copies of the OV alleles released at BWRA, 25 of 288 (8.7%) tadpoles genotyped in 2004 contained copies of the unique alleles. These 25 tadpoles only allow for assignment of a minimum of 15 movements through the treatment arrays however, as the presence of multiple tadpoles with alleles from OV in a single tank (N=6) may simply be multiple progeny resulting from a single movement by an adult previously released as a tadpole in 2003 or 2004. Four tadpoles contained the unique alleles at both loci and two of the three central tanks had tadpoles that contained OV alleles.

We assumed that each movement consisted of a straight-line path between a central pond and the peripheral pond from which the offspring were collected. The greatest number of dispersal events were recorded from the 180 m forested treatments (N=7), followed by four movements through the 60 m field treatments, and two each from 60 m forested treatments and 180 m field treatments. The genetic data alone provide some information regarding the interaction of habitat and distance on movements of gray treefrog juveniles post-metamorphosis (G_{Int}=3.018, df=1, P=0.082).

Dispersal of toe-clipped individuals

We marked a total of 545 metamorphosing treefrogs in 2002 and 2003. The average size (i.e., snout to vent length) of metamorphosed individuals was 16.4 mm (±0.08 SE) measured prior to the 3rd day post-metamorphosis in most instances. Of the individually marked juveniles, 6 (1%) were recaptured in 2003 and 2004. The majority of movements occurred within forested treatments, with three occurring in 180 m treatments and two occurring within 60 m treatments. Only one movement occurred within field treatments (180 m). When considered alone, juvenile mark-release/recapture data did not display an interaction effect ($G_{\mathrm{Int}}=0.876$, $df=1$, $P=0.349$), but we found a moderate effect between habitat and distance when genetic data were included ($G_{\mathrm{Int}}=3.142$, $df=1$, $P=0.076$; Fig. 2A). In single effect tests, juvenile mark-release/recapture data display a significant relationship when analyzed alone ($G_{\mathrm{Hab}}=3.67$, $df=1$, $P=0.029$), or in combination with genetic data ($G_{\mathrm{Hab}}=7.68$, $df=1$, $P=0.006$), suggesting that juveniles avoid old-field habitat. We found no significant effects of distance on juvenile movement in either case at this scale. None of the toe-clipped juveniles were recaptured at their natal ponds after the initial season in which they were marked, but in several instances tadpoles containing unique alleles were present in the central ponds.

We captured and marked 409 individual adult treefrogs (with 322 recaptures) at the cattle tank ponds during the breeding seasons of 2003 and 2004. Of these, 69 were females (3 recaptures) and 340 were males (319 recaptures). Of the 322 recaptures, we recorded 26 movements (8%) between adjacent cattle tanks by 22 different individuals. Two adult movements were recorded within the 180 m field treatments and nine were recorded from 180 m forest treatments. Eight movements were recorded in 60 m field treatments and seven were recorded from 60 m forest treatments. Considering all adult movements, habitat type and distance interact to influence movement patterns (G_{Int}=5.026, *df*=1, *P*=0.025; Fig. 2B), with distance (G_{Dis}=8.58, *df*=1, *P*=0.008) having a greater effect on movements than habitat (G_{Hab}=5.49, *df*=1, *P*=0.069).

Eighteen of the 26 adult inter-pond movements (69%) were recorded within a single breeding season. Most movements occurred within forest treatments (*N*=6 each), an additional five were recorded from 60 m field treatments, and only one individual moved through 180 m field treatments. Movements within breeding seasons show a slight interaction between habitat type and distance (G_{Int}=3.316, *df*=1, *P*=0.069; Fig. 2C), however there was a stronger effect when testing distance alone (G_{Dis}=7.39, *df*=1, *P*=0.008), than habitat (G_{Hab}=4.66, *df*=1, *P*=0.071). Only eight adult movements were recorded among breeding seasons, with one recorded from 180 m field treatments and three from the 180 m forest treatments. We recorded an additional three from the 60 m field treatments and one from 60 m forest treatments. Among-season adult movements showed only a slight interaction effect (G_{Int}=3.062, *df*=1, *P*=0.080) and no significant single treatment effects. Similarly, only slight interaction effects were detected when considering all juvenile and adult among season movement data (G_{Int}=3.645, *df*=1, *P*=0.056; Fig. 2D). Of 44 adult individuals recaptured among seasons, breeding pond philopatry was observed from 35 individuals (79.5%). When all movement data (i.e., within- and among-seasons, adults and juveniles) as recorded by mark-recapture and genetic data were combined, we observed a significant interaction effect of distance and habitat (G_{Int}=5.108, *df*=1, *P*=0.024; Fig. 3).

DISCUSSION

Subsequent to Wright (1940) and Andrewartha and Birch (1954), the ‘evolution’ of the metapopulation paradigm began with MacArthur and Wilson’s (1967) and Levins’ (1969) models regarding the dynamics of extinction and colonization as they pertain to the distributions of multiple species and populations of single species, respectively. In the years that followed, more complex metapopulation models included aspects not originally parameterized in the simpler early models (as reviewed by Vandermeer and Carvajal [2001]), and focused on aspects of habitat patch morphology (i.e., size, shape) and configuration (i.e., relative position in the landscape). However, even the more complicated models assumed matrix habitat composition to be unimportant to landscape-level population dynamics (Vandermeer and Carvajal 2001).

Increasingly, studies of metapopulations are considering the resistance of matrix habitat to movements between habitat patches (Aberg et al. 1995; Sisk et al. 1997; Pither and Taylor 1998; Gascon et al. 1999; Ricketts 2001; Marsh et al. 2004). Because the relative magnitude of dispersal among subpopulations has large impacts on extinction probabilities (Hanski et al. 1995; Hanski 2000), and by definition occur via matrix habitat, it is logical that the variation in the composition of matrix act in concert with other aspects of metapopulations, such as inter-patch distance, to affect metapopulation dynamics.

Interaction of distance and matrix

We found a significant interaction effect between matrix habitat type and distance treatments, when considering all movements that occurred among experimental ponds during all periods of the year and all age classes of individuals. Our results indicate that as inter-pond distance increases, non-forested matrix represents more of a barrier to movements. That this increasing matrix resistance-by-distance effect on amphibians is apparent at these relatively short distances, suggests that the negative impacts of habitat destruction between populations can be substantial. In our study, the presence of matrix had no effect on overall movements between ponds separated by 60 m, but ponds separated by 180 m experienced a 74% decrease in inter-pond movements. These data are of crucial importance in the face of increasing conversion of forested habitat for agriculture and urbanization. As forested habitat fragments dwindle in number and size, the distances between adjacent fragments increase. Combined with increasing harshness of intervening matrix, successful dispersal among sites become increasingly difficult, and the influx of 'rescuers' that seem to be typical of natural amphibian populations (Gill 1978; Sinsch 1992a) may be insufficient to maintain adequate local population sizes.

Distance effects

Although we detected a significant interaction among inter-pond distance and matrix composition, adult treefrog movements appear more affected by breeding pond isolation than by habitat type. Other studies have found that adult forest-associated amphibians can withstand brief periods of activity within short spans of non-forested habitat (e.g., Marsh et al. 2004), and aspects of movement behavior may help explain this ability. For example, most movements occur nocturnally when the effects of direct sun exposure would be alleviated, and are associated with rainfall events which would further help prevent desiccation (e.g., Chapter 2B). Directed, nighttime movements should be possible through non-forested habitat types even at great distances given appropriate environmental conditions, as is evident from numerous examples of long distance amphibian dispersal events reviewed in Smith and Green (2005). Similarly, Marsh et al. (2004) found that *Plethodon* colonize artificial forested habitat across short distances of field habitat, although Rittenhouse and Semlitsch (*in review*) found behavioral avoidance of habitat edges in *Ambystoma*. The observed effect of isolation on inter-pond movements most likely represents a behavioral affinity for individuals to remain near previously successful breeding locations (i.e., philopatry without restricting activity to a single pond), rather than result from physiological or morphological limitations.

Matrix effects

We found that juvenile movements are more restricted by habitat type than by distance. Significantly fewer movements were recorded from juveniles traveling through old-field habitat than through forest when assuming straight-line movements. Many studies of amphibian migration and dispersal have reported approximately straight-line movement path among seasonal resources (e.g., Carpenter 1954; Shoop 1965, 1968; Semlitsch 1981; Kusano et al. 1995; Madison 1997), and juveniles have been thought to be the primary dispersal agents of many pond-breeding amphibian species (Jameson 1956; Gill 1978; Breden 1987; Berven and Grudzien 1990; Reading et al. 1991; Sinsch and Seidel 1995; Sinsch 1997), given the proclivity for philopatry as adults (Gill 1978; Sinsch 1992b;

Driscoll 1997, 1998). Juveniles may be “programmed” to disperse great distances to locate novel breeding habitats, but morphological and physiological restrictions may limit a juvenile’s ability to traverse sub-optimal habitat types (Goater et al. 1993; Joly et al. 2001). For example, long distance dispersal by a recently metamorphosed treefrog would take longer than a similar distance by an adult simply because of differences in size. Therefore, longer duration movements through non-forested habitats result in a greater probability of changes in microclimate that would negatively affect, and potentially kill the disperser. Furthermore, juveniles have a high surface area to volume ratio and desiccate more rapidly than larger adults (Rothermel and Semlitsch 2002), which may also limit the ability to traverse certain habitat types. Lastly, juveniles may be unable to detect appropriate habitat at distances as great as adults (Rothermel and Semlitsch 2002; Rothermel 2004), leading juveniles to either avoid inhospitable habitat (Rittenhouse et al. 2004) that spans even short distances, or dooming them to traverse matrix habitat without adjacent forest.

Our results are similar to those of Joly et al. (2001), who found that matrix habitat is important in determining landscape-level pond occupancy rates for newts. However, studies elucidating the negative effects of matrix on dispersal seem at odds with reports of frequent population turnover from rapid recolonization following local extinctions, even in highly fragmented landscapes (e.g., Hecnar and M’Closkey 1996; Skelly et al. 1999; Carlson and Edenhamn 2000). This apparent contradiction can be reconciled if we consider rates of dispersal within fragmented landscapes in context with the temporal span that has elapsed between fragmentation and observed patch occupancy. As described in Hanski (2000), selection acts against dispersal following fragmentation because of the increased risks associated with movements between patches, such as increased matrix harshness or inter-patch distance. The result is a reduction in movements between patches, and an increase in the extinction probability of local populations. An increase in local extinctions results in more numerous empty habitat patches, and subsequent selection at the regional scale for an increase in dispersal. The ability of populations to respond to increased selection for dispersal following habitat fragmentations depends on the frequency of successful dispersal events (i.e., those that result in an increase in reproductive fitness) within the metapopulation. Therefore, rapid colonization of empty pond-breeding amphibian habitats may represent an ability to cope with habitat fragmentation at some scale.

On the other hand, species that are adapted for frequent movements among patches may have an increased risk of local extinction in newly fragmented landscapes than those that have limited dispersal, because of the propensity of individuals to attempt movements through matrix that has higher risk of mortality (Gibbs 1993; Hanski 2000; Marsh et al. 2004). Taxa that have evolved to inhabit highly ephemeral habitats, such as many pond-breeding amphibians, exhibit high dispersal rates to compensate for large deterministic or stochastic local extinction rates (Hanski 2000). Therefore, they may be at increased risk of extinction due to habitat fragmentation, despite apparent high rates of colonization of empty patches immediately following fragmentation of continuous habitat. Furthermore, population dynamics can be affected by the number of dispersers to a site (i.e., ‘rescuers’), not just the probability of a dispersal event (Hanski et al. 1995; Hanski 2000). Thus, observed amphibian population turnover may be deceiving, in that we simply are not detecting any immediate observable effects of fragmentation on overall colonization ability or occupancy rates, even though the magnitude of connections among sites has diminished.

Pond-breeding amphibian metapopulations

With increasing numbers of studies on amphibian metapopulations, issues have arisen regarding the applicability of classical metapopulation models to amphibians in general. First, the importance of terrestrial habitat adjacent to breeding ponds has elucidated the inadequacy of the 'ponds as patches' view of amphibian populations (Marsh and Trenham 2001), and the capacity for colonization of new ponds within a breeding season (Marsh et al 1999; Chapter 1) further blurs the designation of patch boundaries. Second, population turnover events have proved difficult to elucidate for some amphibians (but see Sjögren 1991; Sjögren-Gulve 1994; Sinsch and Seidel 1995; Hecnar and M'Closkey 1996) and in some instances all possible sites within a region are occupied (Gill 1978; Sinsch 1992a). These latter observations and others (Gill 1978; Easteal and Floyd 1986; Reimchen 1990; Sinsch and Seidel 1995; Carlson and Edenhamn 2000; Pechmann et al. 2001; Lehtinen and Galatowitsch 2001), suggest that ponds may be too rapidly recolonized to detect absences, or that many habitat patches remain in a perpetual state of 'rescue' from adjacent 'source' sites (Gill 1978; Gibbs 1993; Sinsch and Seidel 1995; Sinsch 1992a, 1997). Third, long distance movements are difficult to detect in traditional (e.g., mark-recapture/release) studies and often a small number of individuals can be expected to disperse great distances (Smith and Green 2005). While the importance of rare long distance movements is evident from a genetic standpoint (i.e., maintenance of allelic diversity), the scale and abundance of movements that are most important to population demographics has yet to be determined. Nonetheless, some amphibians may be more vagile than previously believed.

Our data provide information regarding a number of these issues. Experimental ponds were filled during the fall of 2002, and by the spring of 2004, all were colonized, suggesting that ponds were discovered as animals moved from overwintering sites to their usual breeding site(s). This holds with the results of Johnson and Semlitsch (Chapter 1) who found rapid colonization artificial breeding sites surrounding natural *H. versicolor* breeding ponds. These data support the contention that at least some pond-breeding amphibians have the capability to rapidly colonize sites and occupy all patches within a landscape (Gill 1978; Sinsch 1992a; Carlson and Edenhamn 2000; Lehtinen and Galatowitsch 2001; Pechmann et al. 2001). Further, the lack of an effect of distance on juvenile movements supports the notion that post-metamorphic dispersal from natal ponds is a primary mechanism contributing to observed 'rescue' effects among amphibian subpopulations (Gill 1978; Gibbs 1993; Sinsch and Seidel 1995; Sinsch 1992a, 1997). And adults (or at least males) tend to be more philopatric (Ritke et al. 1991; Sinsch 1992b), as evident from the significant effect of distance on inter-pond movements. Considering only information regarding frequency of movements across different distances, these data suggest that if breeding ponds are separated at the scale of normal migration distances, patchy population dynamics (i.e., high levels of movement among sites and low levels of stochastic local extinctions) may best model natural populations of pond-breeding amphibians. However, by incorporating the effects of inter-pond habitat composition on movements, we provide a clearer picture regarding interactions among sites in realistic (i.e., fragmented) landscapes. Our data suggest that in breeding-pond associations that contain extensive non-forest matrix, movements among sites can be diminished, and amphibian populations may shift from the realm of 'patchy' population dynamics to that of traditional metapopulations.

Conclusions

Movements are one of the most studied aspects of individuals and one of the most important processes from a population or metapopulation standpoint, yet factors influencing movements from one population to another are poorly understood. Direct methods of monitoring the frequency of dispersal events and the distances at which they occur are limited by the extremely large sample sizes and monitoring areas needed to observe rare long-distance movements. Our study utilized aspects of both mark-recapture and genetic techniques to boost our ability to detect movements in an experimental system devoid of the historical biases of pre-existing patch associations.

We have found that the scale of forested habitat fragmentation has the capacity to exert increasingly negative effects on the natural dynamics of pond-breeding amphibian populations. Breeding area philopatry reduces the frequency of adult movements among sites, but perhaps more importantly, alteration of forested habitat decreases the ability of juveniles to disperse. Many pond-breeding amphibians breed in ephemeral aquatic habitat to avoid aquatic predators. As a result, yearly reproductive success is highly variable, and depends on the local environmental conditions that affect pond hydroperiod (Semlitsch et al. 1996). 'Hotspot' or 'source' populations that provide immigrants that 'rescue' other populations from extinction, are an important component of pond-breeding amphibian population dynamics (e.g., Gill 1978; Sinsch 1992a). It is critical to maintain adequate habitat connections among populations because pond breeding amphibians are adapted to disperse frequently among sites, and because the location of the 'hotspot' in the landscape is temporally variable, thus the location of dispersers that are essential for long-term population persistence are unpredictable.

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Figure 1. Field-site schematic diagram, with starred circles denoting central cattle tank locations and open circles representing peripheral tanks. Lighter shades represent old-field habitat, and dark areas signify deciduous forest.

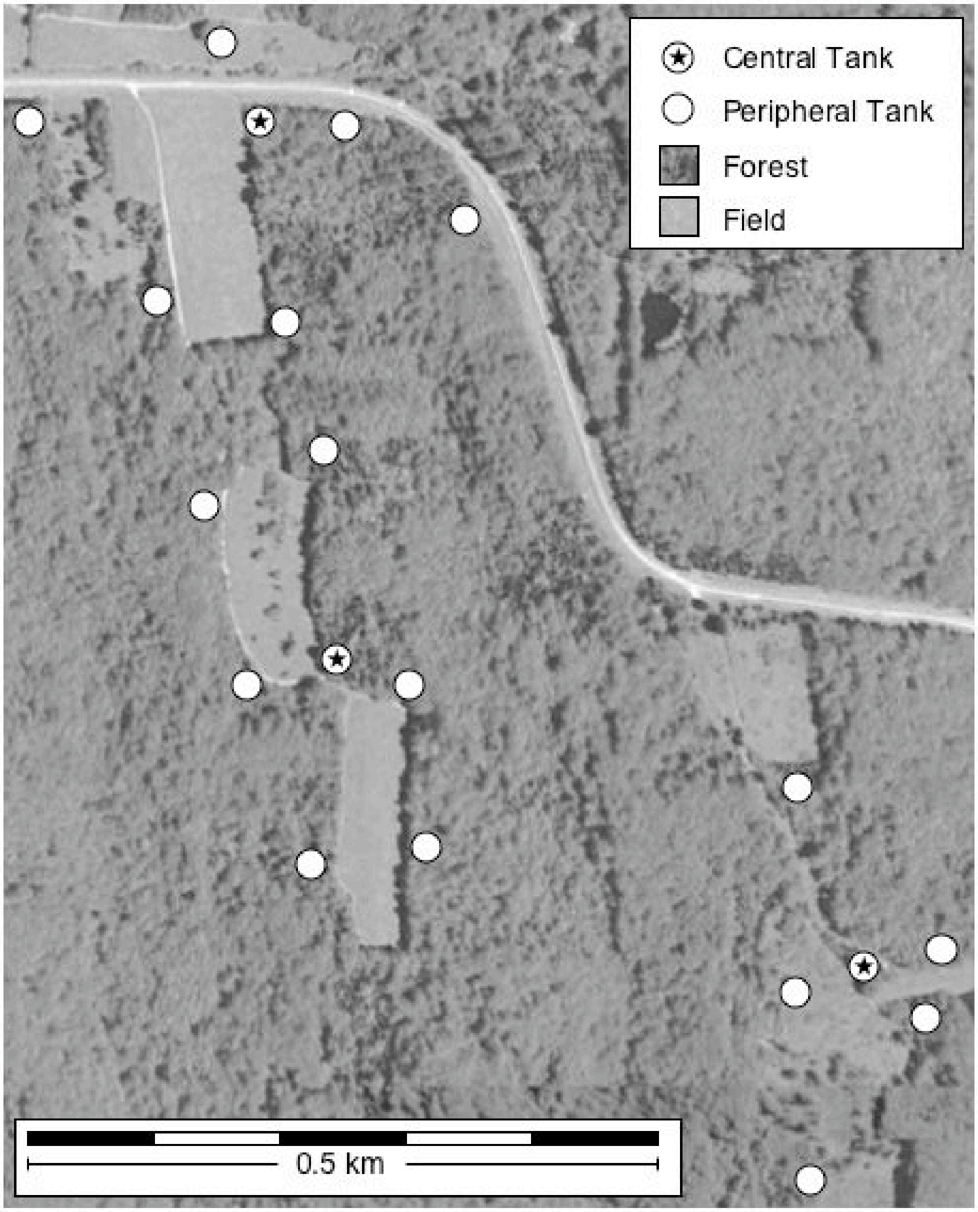

Central Tank
Peripheral Tank
Forest
Field
0.5 km

Figure 2. The interaction of habitat type and distance for **A)** juvenile, **B)** adult, **C)** within-season, and **D)** among-season movements. Juvenile movements include both mark-release/recapture and genetic data. Among-season movements include adult and juvenile mark-release/recapture and genetic data.

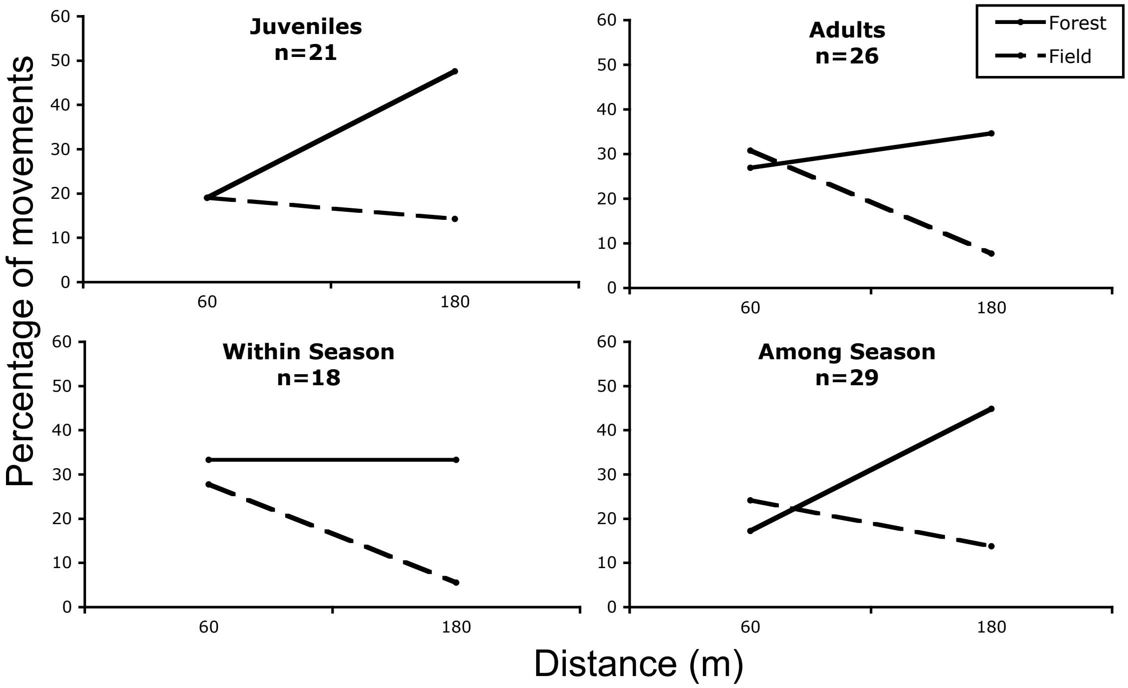
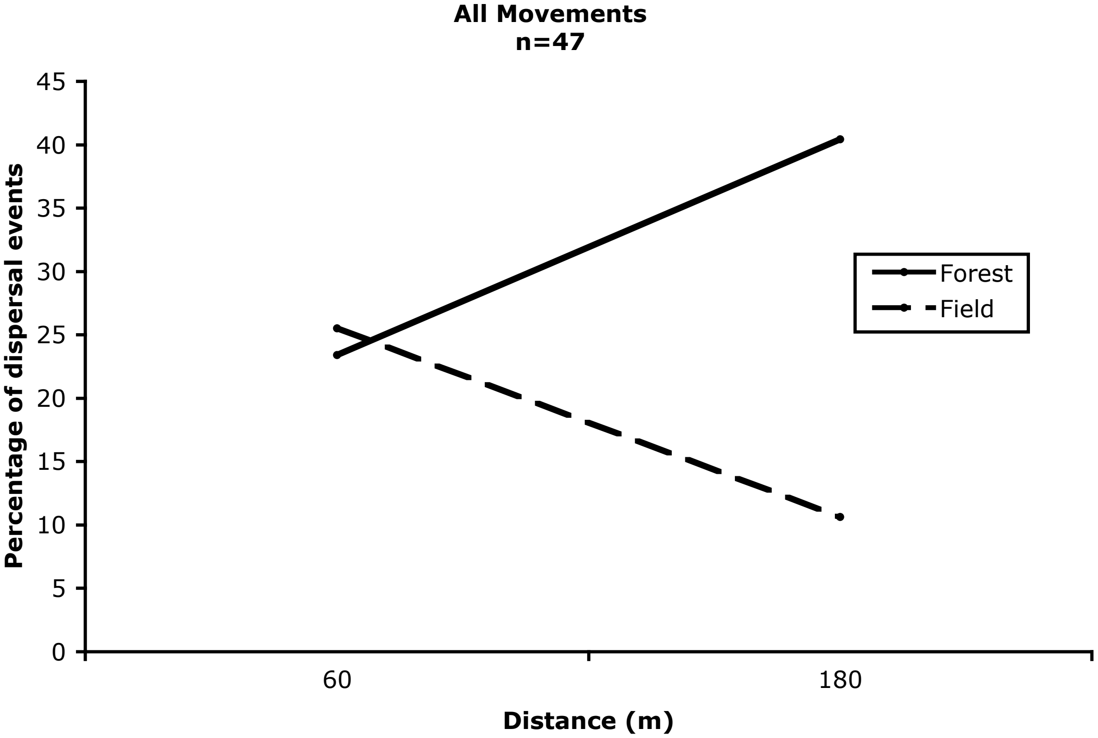

Percentage of movements
Juveniles
n=21
Adults
n=26
Within Season
n=18
Among Season
n=29
Forest
Field
0
10
20
30
40
50
60
60
180
Distance (m)

Figure 3. Interaction of habitat type and distance for all movement data.


All Movements
n=47
45
40
35
30
25
20
15
10
5
0
Percentage of dispersal events
60
180
Distance (m)
Forest
Field

Chapter 4

MICROSATELLITE INHERITANCE AND FINE-SCALE POPULATION STRUCTURE OF THE TETRAPLOID GRAY TREEFROG (*HYLA VERSICOLOR*)

Jarrett R. Johnson

ABSTRACT

The maintenance of connections among populations is important for limiting the risk of local or regional extinction. In increasingly urbanized and modified landscapes, the capacity for natural population dynamics to persist is limited and information regarding the importance of movements at multiple scales is critical. Dispersal movements among distant sites are difficult to measure with direct methods, and estimates of gene flow can provide information regarding the frequency and scale of inter-pond dispersal events. I used three microsatellite loci to estimate the mode of inheritance, degree of population differentiation, and extent of population structure of the tetraploid gray treefrog, *Hyla versicolor*. Ten families of treefrogs were assessed with quantitative and qualitative genetic techniques to determine the mode of inheritance, and tadpoles from twenty breeding sites separated by distances ranging from 0.1 to 73 km were sampled to estimate gene flow among populations. Conclusions regarding the mode of inheritance were hindered by low sample sizes, but in general a tetrasomic model of inheritance was most parsimonious. Populations separated by greater than 30 km displayed significant

pairwise genetic estimates of population differentiation (F_{st}) with a greater frequency than those separated by distances less than 30 km. Populations separated by less than 3 km showed a lack of differentiation, although not in all cases. Overall, I observed a positive correlation between geographic and genetic distance among sites. I conclude that regional associations of treefrog populations vary in expected patterns of metapopulation dynamics based on the scale of inter-pond distances, with 'patchy' metapopulations existing when ponds are nearby (i.e., <3 km), and more 'classical' metapopulation dynamics prevailing as inter-pond distance increases (i.e., 3-30 km).

INTRODUCTION

Species typically have spatially uneven or patchy distributions across their ranges, even in relatively undisturbed natural habitat (e.g., Wright 1940; Andrewartha and Birch 1954). Thus, the study of the probability and rate of exchange of individuals among populations has long been of interest to ecologists and population biologists. But the importance of information regarding dispersal has become increasingly important to the field of conservation biology, as expanses of continuous natural habitat are fragmented by agriculture or urbanization (e.g., Pulliam 1988; Hanski and Gilpin 1991; Fahrig and Merriam 1994). Connections among populations are important determinants of both demographic and genetic aspects of populations (e.g., Hanski 2000; Trenham et al. 2001). For example, in the absence of connections among populations (i.e., complete isolation) local populations suffer increased risk of extinction due to lack of 'rescue' effects via immigration and a reduction in genetic diversity due to random fixation of alleles via drift. Further, once a population is extinct (or an allele is fixed), isolated populations have no prospects for recolonization (or influx of new alleles) by dispersal of individuals from adjacent sites. Therefore, protection of inter-population movements is of critical importance to the persistence of species and the preservation of overall biodiversity.

The interaction of populations within a landscape has been modeled in a variety of forms depending upon the degree of movements among local populations and the frequency of local extinctions (Levins 1970; Hanski and Gilpin 1991; Harrison 1991). When inter-population movements are frequent and local extinctions are rare, each subpopulation is considered part of an overall 'patchy' population, and differentiation (genetic or otherwise) among populations should be limited. From a genetic standpoint this means that drift would be a relatively benign evolutionary force compared to the homogenizing effects of gene flow. When movements among populations are extremely limited, differentiation of local populations is expected to be high, with genetic drift and possibly inbreeding depression leading to reduced genetic diversity and increased local extinction risk. Further, when dispersal among sites is limited, the risk of extinction at the landscape level is increased because recolonization of suitable unoccupied locations does not occur. Between these two extremes exist a variety of metapopulation models in which movements among sites are frequent and local population turnover is high due to recolonization of sites following local extinction. Metapopulation dynamics and the resulting genetic consequences vary among systems of populations depending on aspects of the local populations. For example when 'source-sink' dynamics are prevalent, 'source' sites are more likely to produce dispersers than others, and the extinction probability of the 'source' population is low relative to the 'sinks.' Generally 'source' populations are considered to contain a greater number of individuals and therefore experience a reduced extinction risk relative to ‘sink’ populations, but this does not have to be the case. Regardless, genetic differentiation could be great among ‘sink’ populations, but insignificant among comparisons of the ‘source’ population with each ‘sink.’

Amphibians are typically considered to be a particularly suitable group of organisms in which to assess inter-population movements based on aspects of their natural history (e.g., limited movement capability, congregation at breeding sites). However, assessment of population structure, and the ultimate evolutionary implications of observed movement patterns are relatively rare. Furthermore, reports of long-distance amphibian movements from mark/release-recapture studies are increasing in the literature and seem to contradict previous studies using molecular markers that supported the hypothesis of limited movement capability. While the spatial and temporal scale of most studies of genetic structure is much greater than can be achieved via mark/release-recapture techniques, and the results of each type of study are usually not directly comparable, some studies have found fine-scale genetic structure of amphibians (e.g. Newman and Squire 2001).

Rare long-distance dispersal movements can have relatively large effects on genetic variation and the degree of differentiation among populations even when the demographic ‘rescue’ effects of immigration by dispersers are not realized. Similarly, inter-population dispersal movements can affect the genetic diversity of subpopulations differently depending on population size and the frequency of the exchange of individuals. Therefore, the effect of dispersal on the evolution and persistence of species depends upon the scale and magnitude at which connections among subpopulations are maintained. Observed patterns of genetic differentiation can therefore be used to test hypotheses regarding the type of population dynamics occurring among populations at a regional scale.

The objective of my study was to test for differentiation among gray treefrog breeding sites and identify the scale at which gene flow commonly occurred. Additionally, I aimed to detect landscape features that restricted movements between sites, and relate observed genetic similarities among sites to direct estimates of gray treefrog movement capabilities. Ultimately, I used the correlation of genetic distance estimates and geographic distances among sites to determine the most likely model of regional population dynamics for gray treefrogs and pond-breeding anurans in general.

METHODS

Study Species

The gray treefrog (*Hyla versicolor*) is a sexually reproducing autotetraploid species with multiple origins from its diploid progenitor, *Hyla chrysoscelis*. Previous studies have estimated at least three separate origins of *H. versicolor* occurred between 1 and 4 m.y.a. (Ptacek et al. 1994). The relatively recent origin of the tetraploid lineages suggests that much of the genome should be inherited tetrasomically (see below), but both tetrasomic and disomic inheritance has been observed at isozyme loci (e.g. Marsden et al. 1987). Despite the difficulties of scoring allele dosages of autotetraploids, *H. versicolor* was chosen due to high local abundances, and because they are distributed throughout the central portion of the state (Johnson 2000).

Polyploids provide a particularly intriguing opportunity to investigate fine-scale population differentiation because of the capacity for high degree of genetic variation owing from the duplication of an entire diploid genome. In tetraploids formed by the hybridization of two parental species (i.e., allotetraploids), the increase in genetic variation is immediate because the genome of one parental species operates somewhat independent from the other. On the other hand, while autotetraploids also have twice as many copies of each gene as the diploid parental species, regulation of gene dosage typically silences the extra gene copies and overall genetic diversity remains similar to that of the progenitor species. Silenced genes are effectively freed from selection against mutation and over time accumulate structural changes that progressively 'diploidize' the genome. While 'diploidization' essentially occurs immediately in allotetraploids, and therefore they can be analyzed as diploids, studies of autotetraploid genetics need information regarding overall divergence time and inheritance patterns of the loci to be used for generation of population differentiation estimates. For this reason, I performed a study of inheritance patterns in *H. versicolor* using three microsatellite loci.

Inheritance patterns

For a separate experiment (Chapter 3), I captured 10 pairs of gray treefrogs in amplexus at a single breeding site during the summers of 2002 and 2003. Each pair deposited fertilized eggs in separate containers in the laboratory and then were toe-clipped for identification upon recapture, and released at the breeding pond from which they were collected. The excised toes from each parent and a subset of their tadpole offspring (N≅30) were collected and stored in individual tubes. Each family group was genotyped following the same extraction, PCR, and scoring procedures as described below.

Allele dosages were calculated by generating binomial 95% confidence intervals for allele transmission frequencies (i.e., the ratio of offspring containing an allele and the number of total offspring genotyped; Rodzen and May 2002). If allele transmission frequencies ±95% confidence intervals overlapped 0.50 then the parental genotype contained only a single copy of the allele (for either disomic or tetrasomic inheritance), and if allele transmission frequencies ±95% C.I. overlapped 0.83 then the parental genotype was estimated to have contained two copies of the allele (Figure 1). Transmission frequencies of 0.00 were assumed to occur due to small sample size and were assigned an estimated dosage range of 0-1. Similarly, transmission frequencies of 1.00 were assigned an estimated dosage range of 3-4 (Table 1).

Using the estimated allele dosages for parental genotypes calculated from offspring transmission frequencies (Table 1), I constructed complete genotypes for all parents that could not be ascertained through gel scoring procedures alone (Table 2). This method is preferable to estimating dosage from observed band intensities because each PCR reaction can vary greatly in terms of relative amplification of each allele that comprises an individual’s genotype. Each parental cross was then qualitatively analyzed using the complete genotypes of each parent, and the most probable mode of inheritance at each locus was determined. Further, qualitative analysis of inheritance data provides insight regarding the frequency of double reduction (Figure 2), or the presence of null alleles.

Sampling

The central location of sampling for this study was the Baskett Wildlife Research Area (BWRA) in central Boone County, Missouri. From May to August 2000, I collected between 35 and 50 treefrog tadpoles from nine breeding ponds from within BWRA (Figure 3). Additional sampling locations were determined based on the location of intersections among concentric rings of increasing radii (10, 20, 30, 40 km) around the BWRA and transects extending outwards in four directions (Figure 3). I collected gray treefrog tadpoles from breeding ponds located in the proximity of the intersection points at each distance (N=13), for a total of 22 sampling locations.

Tadpoles were collected via dip-netting along the perimeter of each pond, and haphazardly selecting a subset of tadpoles captured within each sweep of the net. Tadpoles comprising the total sample to be genotyped were collected from at least 5 locations along the edge of ponds. Because gray treefrog are prolonged breeders (Johnson 2000), I sampled each pond at least twice during the summer to ensure adequate representation of genotypes from individuals that bred early in the season and those that bred later. Haphazard selection of different size classes of tadpoles from each dip net sweep further increased the probability of acquiring a representative sample of each treefrog population's alleles. Tadpoles were briefly anesthetized in a 0.04% solution of ethyl-*m*-aminobenzoate methanosulfonate (MS-222) and placed in individual 1.5 μL microcentrifuge tubes filled with 95% EtOH.

Extraction, PCR, and scoring

Twenty-five milligrams of tadpole tail tissue was lysed with Proteinase K and digested for 12 hrs at 55 °C as per the protocol provided with Qiagen DNEasy DNA extraction kits. Following lysis and extraction, I obtained DNA in concentrations of approximately 40ng/μL. Samples were stored frozen (-80 °C) in Buffer AE (Tris/EDTA) provided in the DNEasy kits. Krenz et al. (1999) developed microsatellite loci for diploid gray treefrogs (*H. chrysoscelis*), but neither published PCR primer sequences nor screened their loci to determine relative levels of polymorphism. I designed primers from diploid gray treefrog DNA sequences published in GenBank using PrimerSeq from the DNAStar software package, and screened the loci developed by Krenz et al. (1999) on tetraploid gray treefrogs. Ultimately, I found three (19F9G, 6M4E, 6M8C; Table 3) diploid gray treefrog loci that reliably produced clean, clear allelic banding patterns for tetraploid gray treefrogs. Two of these loci have been used in at least one other study of tetraploid gray treefrog microsatellites (Espinoza and Noor 2002).

PCR recipes were as follows: 5 min denaturing at 95 °C, followed by 45 cycles of 1 min denaturing at 95 °C, 30 sec at specific primer annealing temperatures (Table 3), and 1 min extension at 72 °C, concluded by a final extension for 5 min at 72 °C. PCR reactions were performed in 0.2 ml tubes with a total reagent volume of 12.5 μl, and mineral oil overlay. I used 20 ng of template DNA in each reaction, and the final concentrations of reagents in the total volume are as follows: 1X buffer, 0.4-0.8 mM dNTPs, 0.4μM forward and reverse primers, and 0.5-1 U Taq Polymerase. Forward primers were 5' labeled with either FAM, TET, or HEX fluorescent labels (Table 3) for automated allele scoring. All samples were sent to the University of Missouri's DNA

Core Facility for size scoring, and I binned alleles into categories using ABI Prism Genotyper Software.

Analysis

For calculation of genetic distance estimates, individual genotypes were scored using a binary code (0/1) representing presence or absence of alleles of each size (e.g. Mengoni et al. 2000). Genotypes were treated in this manner because of the difficulty in accurately distinguishing among each combination of diallelic and triallelic conditions for loci inherited tetrasomically (Espinoza and Noor 2002). Each individual is therefore represented as a string of zeros and ones corresponding to the total number of alleles detected across all three loci. This method of analysis is similar to RFLPs or other dominant genetic loci and has been justified for use with microsatellite loci in other studies of polyploids in taxa including alfalfa (Mengoni et al. 2000) and sturgeon (Rodzen and May 2002). Using Arlequin Software for Population Genetics Data Analysis Version 2.0 (Schneider et al. 2000), I calculated pairwise F_{st} (Wright 1969) via analysis of molecular variance (Weir and Cockerham 1984), and conducted Mantel's tests to relate geographic distance to genetic distance estimates (Rousset 1997; Hutchinson and Templeton 1999).

RESULTS

Inheritance

The sample sizes required to obtain transmission frequency estimates with 95% confidence intervals that are small enough to distinguish between the theoretical expectations of 0.5, 0.83, and 1.00, and are difficult to achieve in studies of inheritance (Marsden et al. 1987, Rodzen and May 2002). As a result of low sample sizes due to the need to maximize the number of tadpoles from each clutch used for a separate experiment (Chapter 3), allele transmission frequencies were ambiguous and overlapped multiple theoretical expectations in some cases (Table 1). Despite this lack of resolution, allele transmission frequencies were congruent with observed patterns of inheritance for all treefrog pairs and loci as illustrated by the example in Table 3. Locus 6M8C was omitted from inheritance analyses due to a high frequency of shared alleles between parents, making accurate assignment of dosages difficult and thus complete genotypes are not included in Table 4.

In four instances, both parental and all offspring genotypes within a family contained only two distinct alleles at 19F9G. Only once were both parents homozygous for the same allele and produced completely homozygous offspring at 6M4E. In the remaining cases (N=15), a tetrasomic mode of inheritance was the most parsimonious with observed patterns of allele transmission from parent to offspring at the loci 19F9G and 6M8C (Table 4). This conclusion is based primarily upon allelic combinations that excluded the possibility of two independently segregating disomic loci when assuming that similarly sized alleles comprised each locus (Table 4). However, the presence of null alleles at these loci would allow the observed patterns of inheritance to occur from two disomic loci in all cases except one (Table 4).

Genetic Variation and Population Differentiation

Two of the three loci used in this study displayed large amounts of variation (Table 5), and in all but one instance, the highest diversity within a site was recorded from locus 6M4E. The estimates of gene diversity presented in Table 5 can be interpreted as the likelihood of a randomly drawn individual possessing a heterozygous genotype. Large values (i.e., close to 1.0) indicate a large frequency of heterozygotes in the population at a particular locus. The high degree of gene diversity at 19F9G and 6M4E is also evident from the relatively high number of unique haplotypes within each population at each locus (Table 5). The most common allele for each locus was not consistent among populations, but always represented between 10 and 13% of the total alleles in each population.

Overall, I observed differences in allele frequencies among populations, with a low but significant global F_{st} estimate of 0.06 ($P<0.001$). In general, the magnitude of pairwise estimates of F_{st} were correspondingly low (Table 6), and the highest degree of genetic differentiation recorded was 0.21. The highest single locus estimate of pairwise F_{st} was recoded by the least variable locus (6M8C) and was 0.49. In all, 68 out of 231 (29%) possible pairwise comparisons of genetic divergence were statistically significant ($\alpha=0.01$) with all populations displaying divergence from at least one sampled study site. When all sampling sites at BWRA were lumped, the most isolated population (OV) showed divergence in 12 of the 13 possible pairwise combinations of sampling locations, and 4 other sites (LD, EL, GU, HA) displayed 8 significant pairwise F_{st} values.

I found a significant positive relationship between population differentiation (pairwise F_{st}) and geographic distance (ln[m]) using a Mantel test across all loci (Figure 4; *P*=0.008). The observed relationship fits the expected association of genetic and geographic distances outlined by Hutchinson and Templeton (1999) for regional equilibrium (Figure 4). The strongest isolation by distance effect was evident from locus 19F9G, and was the only locus with a significant relationship from single-locus Mantel tests (*P*<.001).

DISCUSSION

Inheritance

Overall, neither quantitative assignment of allele dosages nor qualitative examination of allele transmission allowed straightforward conclusions regarding the mode of inheritance at these microsatellite loci. In general however, when assuming the absence of null alleles, the data suggested that a tetrasomic model was most appropriate. Conversely, previous work on the inheritance of tetraploid gray treefrogs has indicated varying degrees of diploidization of the genome both among loci and among individuals. Danzmann and Bogart (1982, 1983) found both disomic and tetrasomic inheritance of isozyme loci among individuals, and Marsden et al. (1987) found similar results using similar genetic markers. Isozyme studies of tetraploid inheritance have the advantage of observable allelic dosage and accurate assignment of diallelic and triallelic combinations within an individual's genotype. Further research is required to adequately determine the mode of inheritance at these microsatellite loci in tetraploid gray treefrogs.

Rodzen and May (2002) performed a much larger-scale investigation of polyploid (4N, 8N) sturgeon inheritance patterns and even with more appropriately large sample sizes, were unable to completely resolve the mode of inheritance at microsatellite loci. They proposed that studies of the population genetics of autopolyploids avoid the confusion of assigning genotypes based on assumed modes of inheritance and analyze each allele as a dominant nuclear marker (i.e., RAPD or AFLP). Lynch and Milligan (1994) outline the methods of RAPD (random amplified polymorphic DNA) analyses as they pertain to population genetics. Rodzen and May (2002) discuss the applicability of RAPD analysis techniques to microsatellite markers converted to dominant nuclear haplotypes. Of primary concern was whether a microsatellite allele behaved as a dominant marker, in such that parents containing single copies or two copies of an allele transmit the allele to 50% or 100% of their offspring, respectively. My data regarding single allele inheritance conformed to the expected 50% transmission rates and suggested that conversion of these microsatellite loci into RAPD haplotypes for population genetic analyses was appropriate.

Genetic Variation and Population Differentiation

All of the treefrog populations sampled were polymorphic at each microsatellite locus examined and even when restricting estimation of gene diversity to observed banding phenotypes (i.e., conversion to binary code haplotype) allelic diversity was high. Some studies of diploid amphibian microsatellite loci have found much lower estimates of diversity (e.g., Rowe et al. 1998; Newman and Squire 2001) and the elevated diversity estimates in my study may be the result of the increased ploidy level of *H. versicolor*. Furthermore, ecological aspects of treefrog natural history may work to maintain high levels of genetic diversity. For example, if local extinctions are rare and inter-pond movement of individuals is high (Carlson and Edenhamn 2000), genetic diversity can be maintained despite natural population fluctuations typical of species that breed in ephemeral habitats (Semlitsch et al. 1996).

I found very little population differentiation among populations within the main study site. Interestingly however, two pairs of ponds (WP & TP; TK & HP) separated by short distances showed unexpectedly high levels of divergence (i.e., 10% of ponds separated by <3 m; Fig. 5), as has been observed in other studies of fine-scale population dynamics (e.g. Newman and Squire 2002). Other pairs of ponds separated by the similar distances (e.g., TK & 2P; WP & RP) did not display a similar effect and it is unlikely that these differentiation estimates were the result of long periods of restricted gene flow between the populations. Possibly, the significant F_{st} values were the result of a bias in initial sampling of tadpoles or stochastic fluctuation in allele frequencies due to extreme fluctuations in effective population size. At the landscape scale, I also found a higher degree of genetic similarity among sampling locations than anticipated. Non-significant pairwise F_{st} estimates occurred between sites separated by up to 71 km (RE & PH). Although I did observe a significant positive effect of distance on genetic differentiation, and 50% of all pairwise combinations of sites separated by 30 km or more displayed significant F_{st} values, compared to 20% of pairwise combinations separated by less than 30 km (Fig. 5).

Genetic homogeneity at shorter inter-pond distances suggests a high degree of gene flow among sites. This indicates that movements among sites at the scale of 30 km or less may be frequent, and local extinctions could be rare. However, as Newman and Squire (2002) suggest, the observed lack of fine-scale genetic differentiation could also be the result of extinction-recolonization dynamics if recolonization of extinct sites is accomplished by founder groups with similar genetic make-up. Hutchinson and Templeton (1999) provide the theoretical framework with which to differentiate between these two scenarios and provide insight regarding the nature of treefrog metapopulations.

In the case of regional equilibrium, a positive association between geographic distance and genetic differentiation occurs over all pairwise combinations of sampling locations. The spread of the scatter-plot of such a relationship should increase with geographic distance due to the increased effect of genetic drift as gene flow diminishes. Non-equilibrium situations can be the result of many factors including range expansion or extreme isolation, and would result in a lack of a correlation among genetic and geographic distances. Again, the degree of variation in F_{st} at each distance would depend upon the relative influence of drift and gene flow (i.e., wide scatter in the case of extreme isolation, and narrow scatter in the case of range expansion).

The relationship between geographic and genetic distance observed in my study most similarly resembles the case of regional equilibrium, except that the range of F_{st} is relatively large even when geographic distance is short. That the homogenizing effects of gene flow are not more evident over short inter-pond distances than long ones is a possible illustration of the effect of population fluctuation due to variable breeding habitats (e.g., Semlitsch et al. 1996) even though dispersal limits the frequency of local extinctions. In other words, even when the magnitude of inter-pond dispersal is high, and the allele frequencies between sites are correspondingly similar, the relative effect of drift on allele frequencies is equivalent to the situation in which allele frequencies among populations have diverged.

Despite significant estimates of genetic differentiation at all distance scales examined, I observed significant isolation by distance at distances greater than 30 km. Consideration must be given to the difference between the effect of a single dispersal event between distant populations on genetic variation and demographic processes. While rare dispersal events at great distances can have a homogenizing genetic effect, it is unlikely that the addition of a single individual would provide significant contributions to population size and reduction to extinction risk. Based on genetic data it seems likely that populations separated by less than 3 km of appropriate natural habitat exchange individuals frequently enough to be considered portions of a ‘patchy’ metapopulation, and that populations separated by distances ranging from 3 to 30 km have modest amounts of gene flow that may be representative of more ‘classical’ metapopulation dynamics.

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Table 1. Estimates of allele transmission frequencies for **A)** 19F9G and **B)** 6M4E. Only alleles unique to either parent provide information regarding allele dosages and (--) represent individuals with no unique alleles.

A.

Year	Pair ID	Parent	Allele size (bp)	Offspring allele N	Transmission frequency	95% C.I.	Estimated dosage
2002	OV1	M	179	3	0.429	0.262	1
		F	173	2	0.286	0.239	1
	OV2	M	169	3	0.429	0.262	1
			179	5	0.714	0.239	1 or 2
		F	--	--	--	--	--
	OVB1	M	173	3	0.429	0.262	1
			185	2	0.286	0.239	1
		F	179	4	0.571	0.262	1 or 2
	OVB2	M	179	5	0.714	0.239	1 or 2
			185	4	0.571	0.262	1 or 2
		F	173	3	0.429	0.262	1 or 2
			195	5	0.714	0.239	1 or 2
	OVB3	M	173	7	1.000	0.000	3 or 4
		F	195	5	0.714	0.239	1 or 2
2003	OV1	M	--	--	--	--	--
		F	195	4	0.500	0.247	1
	OV2	M	179	6	0.750	0.214	2
			185	5	0.625	0.240	1 or 2
		F	173	2	0.250	0.214	0 or 1
			195	6	0.750	0.214	2
	OV4	M	173	2	0.250	0.214	0 or 1
		F	179	6	0.750	0.214	2
	OV5	M	173	6	0.750	0.214	2
		F	179	2	0.250	0.214	0 or 1
	OV6	M	173	5	0.625	0.240	1 or 2
			195	8	1.000	0.000	3 or 4
		F	179	7	0.875	0.164	2 or 3

B.

Year	Pair ID	Parent	Allele size (bp)	Offspring allele N	Transmission frequency	95% C.I.	Estimated dosage
2002	OV2	M	144	0	0.000	0.000	0 or 1
			158	7	1.000	0.000	3 or 4
		F	128	3	0.429	0.262	2
			164	0	0.000	0.000	0 or 1
	OVB1	M	158	4	0.800	0.250	2 or 3
		F	134	4	0.800	0.250	2 or 3
			144	2	0.400	0.307	1
	OVB2	M	158	4	0.667	0.269	1 or 2
		F	144	5	0.833	0.213	2 or 3
			176	3	0.500	0.286	1
	OVB3	M	134	6	1.000	0.000	3 or 4
			158	1	0.167	0.213	0 or 1
			176	0	0.000	0.000	0 or 1
		F	144	6	1.000	0.000	3 or 4
			164	1	0.167	0.213	0 or 1
2003	OV1	M	--	--	--	--	--
		F	134	4	0.667	0.269	1 or 2
			164	4	0.667	0.269	1 or 2
	OV4	M	158	6	0.750	0.214	2
		F	144	4	0.500	0.247	1
	OV5	M	148	4	0.571	0.262	1 or 2
		F	164	2	0.286	0.239	1
	OV6	M	134	7	0.875	0.164	2 or 3
		F	164	5	0.625	0.240	1 or 2
			176	4	0.500	0.247	1

Table 2. Summary of allele banding patterns at each locus for each adult treefrog breeding pair collected from OV in 2002 and 2003. Individuals shared too many alleles within pairs to make accurate genotype assignments at locus 6M4E.

			Allele Sizes (bp)											
Year	**Pair ID**	**Sex**	**19F9G**				**6M4E**				**6M8C**			
2002	OV1	M	179	179	195	195	134	134	144	164	165	171	175	--
		F	173	173	195	195	134	134	144	164	175	--	--	--
	OV2	M	169	179	195	195	134	144	158	158	175	179	--	--
		F	195	195	195	195	128	128	134	164	165	175	179	--
	OVB1	M	173	185	195	195	158	158	158	164	--	--	--	--
		F	179	179	195	195	134	134	144	164	165	179	--	--
	OVB2	M	179	179	185	185	134	158	164	164	165	175	179	--
		F	173	173	195	195	134	144	164	176	157	171	175	--
	OVB3	M	169	173	173	173	134	134	158	176	165	175	--	--
		F	169	169	195	195	144	144	144	164	175	179	--	--
2003	OV1	M	173	173	173	173	158	158	176	176	165	175	179	--
		F	173	173	195	195	134	158	164	176	165	175	179	--
	OV2	M	179	179	185	185	164	164	164	164	165	175	179	--
		F	173	173	195	195	164	164	164	164	165	175	179	--
	OV4	M	173	173	195	195	158	158	164	164	165	175	179	--
		F	179	179	195	195	144	144	164	164	165	175	179	--
	OV5	M	173	173	195	195	134	134	148	164	175	179	--	--
		F	179	195	195	195	134	158	158	158	165	175	--	--
	OV6	M	173	195	195	195	134	134	144	158	165	175	--	--
		F	179	179	179	179	144	158	164	176	165	179	--	--

Table 3. Primer sequences, annealing temperatures and number and length of PCR products of three *H. versicolor* microsatellite loci.

Locus	Direction	Sequence	Type	Label	Annealing Temperature (°C)	Product Rang (bp)
19F9G	F	5' GCT AAG GGC CAG ACC AG 3'	$(TG)^n$	TET	62.5	145-191
	R	5' TTT AGT ACA CAG CCA AGG ATG 3'				
6M4E	F	5' ATT TAG TGA TAC ATG CTT AGG 3'	$(TA)^n$	FAM	46	114-186
	R	5' TTT ATT ATC TGC TTA CAT TCA 3'	$(TG)^n$			
6M8C	F	5' CCC TCC CTC ACT TCG TTC 3'	$(AC)^n$	HEX	52	153-195
	R	5' ACT ATA TTG TCT TTG GTG GTA 3'				

Table 4. Examples of procedures for determination of inheritance type of microsatellite loci **A)** excluding the possibility of null alleles, and **B)** including null alleles.

2002 Pair OVB3						
Observed parental genotype:	M	169	173	___	___	
			x			
	F	169	___	195	___	
Observed offspring genotypes:	1	169	173	195	___	
	2	169	173	___	___	*
	3	173	195	___	___	*
	4	173	195	___	___	*
	5	169	173	195	___	
	6	173	195	___	___	*
	7	173	195	___	___	*
Estimated genotypes:	M	169	173	173	173	
			x			
	F	169	169	195	195	
Possible gametes if single tatrasomic locus:	M	3(169/173)				
		3(173/173)				
	F	169/169				
		4(169/195)				
		195/195				
Gametes if two disomic loci:	M	169/173				
		173/173				
	F	169/195				
Observable 4N genotypes:		169	173		[1]	
		169	173	195	[2]	
		173	195		[4]	
2N genotypes:		169	173	195	[2]	

2002 Pair OVB3 with null alleles						
Observed parental genotype:	M	169	___	173	___	
				x		
	F	169	___	195	___	
Observed offspring genotypes:	1	169	173	195	___	
	2	169	173	___	___	*
	3	173	195	___	___	
	4	173	195	___	___	
	5	169	173	195	___	
	6	173	195	___	___	
	7	173	195	___	___	
Genotypes with null alleles:	M	169	000	173	173	
				x		
	F	169	000	195	195	
Possible gametes if single tatrasomic locus:	M	169/000				
		2(169/173)				
		2(000/173)				
		173/173				
	F	169/000				
		2(169/195)				
		2(000/195)				
		195/195				
Gametes if two disomic loci:	M	169/173				
		000/173				
	F	169/195				
		000/195				
Observable 4N genotypes:		169			[0]	
		169	195		[0]	
		169	173		[1]	
		169	173	195	[2]	
2N genotypes:		169	173	195	[2]	
		173	195		[4]	

Table 5. Summary of genetic diversity (i.e., expected heterozygosity) and number of unique haplotypes at three microsatellite loci within 22 *H. versicolor* populations.

		Number of Unique Haplotypes			**Gene Diversity**		
Site	**N**	**19F9G**	**6M4E**	**6M8C**	**19F9G**	**6M4E**	**6M8C**
2P	20	12	18	4	0.9421	0.9895	0.5000
3C	11	7	10	2	0.9273	0.9818	0.3273
EL	18	10	12	3	0.9216	0.9412	0.3072
GU	14	8	9	5	0.9011	0.9341	0.5055
HA	14	8	13	5	0.9231	0.9890	0.7253
HD	19	12	18	7	0.9474	0.9942	0.7135
HP	20	10	16	7	0.8895	0.9789	0.5842
LD	18	8	14	3	0.8693	0.9673	0.4510
LH	3	3	3	3	1.0000	1.0000	1.0000
LP	19	10	16	8	0.8947	0.9825	0.7719
MH	12	5	7	5	0.7576	0.8788	0.6667
MO	13	8	13	5	0.8590	1.0000	0.6282
MP	17	11	16	7	0.9044	0.9926	0.8456
MT	10	9	10	5	0.9778	1.0000	0.7556
OV	19	13	15	7	0.9591	0.9766	0.7953
PH	16	7	13	6	0.8500	0.9667	0.7333
RB	17	13	15	5	0.9632	0.9853	0.5074
RE	15	6	14	8	0.8286	0.9905	0.8286
RP	20	13	17	7	0.9316	0.9789	0.6895
TK	14	11	12	7	0.9670	0.9780	0.8462
TP	18	8	15	6	0.8824	0.9804	0.6797
WP	15	7	13	6	0.8667	0.9810	0.8095
Total	342	38	132	19			
Mean	15.55	9.05	13.14	5.50	0.9074	0.9758	0.6669
SE	0.87	0.58	0.78	0.36	0.0119	0.0058	0.0374

Table 6. Average pairwise genetic distance estimates (F_{st}) over three microsatellite loci (above diagonal) and pairwise geographic distance (km; below diagonal) among 22 *H. versicolor* populations. Bold values of F_{st} indicate significance at α=0.01.

	2P	**3C**	**EL**	**GU**	**HA**	**HD**	**HP**	**LD**	**LH**	**LP**	**MH**	**MO**	**MP**	**MT**	**OV**	**PH**	**RB**	**RE**	**RP**	**TK**	**TP**	**WP**
2P	--	0.00	0.03	0.04	0.06	0.04	0.00	0.05	0.00	0.02	0.04	0.01	0.02	0.03	**0.10**	0.02	0.00	0.03	0.01	0.05	0.01	0.04
3C	12.5	--	0.04	0.06	0.07	0.05	0.00	0.05	0.01	0.04	0.00	0.03	0.04	0.08	**0.12**	0.02	0.00	0.03	0.00	0.06	0.04	0.01
EL	19.8	27.0	--	0.06	**0.17**	0.05	0.05	**0.12**	0.01	**0.08**	**0.11**	**0.11**	**0.11**	0.09	**0.13**	0.07	**0.08**	**0.12**	0.07	**0.09**	0.02	**0.13**
GU	8.4	18.7	25.4	--	**0.09**	**0.08**	0.05	0.05	0.00	0.07	**0.14**	0.01	**0.12**	**0.13**	**0.20**	**0.08**	**0.07**	0.05	0.05	**0.10**	0.03	**0.12**
HA	32.8	28.7	52.5	30.3	--	**0.10**	0.06	0.02	0.04	**0.10**	**0.11**	0.03	**0.09**	**0.13**	**0.19**	**0.11**	0.03	0.06	0.02	**0.13**	**0.10**	**0.09**
HD	29.5	18.4	35.5	36.8	40.7	--	0.04	**0.10**	0.00	0.00	0.06	0.04	0.04	0.00	**0.10**	0.04	0.04	0.07	0.03	0.00	0.02	0.06
HP	0.4	12.9	19.9	8.0	32.7	30.0	--	0.02	0.00	0.05	0.00	0.00	0.00	0.07	**0.15**	0.02	0.02	0.02	0.00	**0.08**	0.03	0.03
LD	18.1	14.9	37.6	17.4	14.9	30.5	18.1	--	0.00	**0.12**	0.10	0.01	**0.09**	**0.16**	**0.21**	**0.09**	**0.06**	**0.07**	0.05	**0.15**	**0.11**	**0.11**
LH	1.1	12.5	18.9	9.3	33.7	29.1	1.4	18.9	--	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.02
LP	2.7	14.5	17.3	9.4	35.5	30.5	2.7	20.8	2.1	--	0.05	0.05	0.04	0.00	**0.12**	0.06	0.03	0.06	0.01	0.00	0.01	0.04
MH	19.1	30.4	30.3	11.7	37.0	48.4	18.7	27.0	19.8	19.0	--	0.07	0.00	0.06	**0.17**	0.06	0.07	0.06	0.03	0.08	**0.09**	0.01
MO	29.2	39.9	39.7	21.4	40.9	58.2	28.8	33.9	30.0	29.2	10.2	--	0.04	0.08	**0.16**	0.03	0.01	0.02	0.01	**0.08**	0.05	0.05
MP	0.5	12.2	19.6	8.9	32.9	29.1	1.0	18.2	0.7	2.7	19.7	29.8	--	0.03	**0.13**	0.01	0.02	0.04	0.05	0.05	0.06	0.02
MT	12.0	10.6	31.4	12.6	21.0	28.3	12.0	6.2	12.8	14.7	23.5	31.8	12.1	--	0.06	0.03	0.03	**0.08**	0.05	0.00	0.03	**0.08**
OV	39.6	28.7	43.0	47.1	49.1	10.3	40.0	40.3	39.0	40.4	58.6	68.4	39.1	38.5	--	**0.09**	**0.08**	**0.16**	**0.12**	**0.09**	**0.13**	**0.17**
PH	35.1	29.0	30.4	43.5	56.1	17.7	35.6	43.6	34.2	34.7	53.7	63.9	34.6	39.5	17.6	--	0.00	0.04	0.04	0.03	0.06	**0.09**
RB	18.5	6.1	30.6	24.8	30.2	12.8	18.9	18.2	18.4	20.2	36.6	46.0	18.1	15.5	23.0	26.0	--	**0.06**	0.00	0.03	0.02	**0.06**
RE	36.0	44.1	50.3	27.6	35.9	62.3	35.6	33.8	36.9	36.9	20.1	13.0	36.5	34.1	72.6	71.0	49.6	--	0.05	0.06	**0.09**	0.03
RP	0.7	12.4	19.2	9.0	33.3	29.2	1.1	18.6	0.4	2.4	19.6	29.8	0.4	12.4	39.2	34.5	18.3	36.6	--	0.04	0.01	0.03
TK	0.5	12.3	20.3	8.2	32.3	29.5	0.6	17.6	1.5	3.2	19.1	29.2	0.8	11.5	39.5	35.3	18.3	35.8	1.1	--	0.03	**0.08**
TP	0.6	12.4	19.4	9.0	33.2	29.2	1.0	18.5	0.5	2.5	19.6	29.7	0.3	12.3	39.2	34.6	18.3	36.6	0.1	1.0	--	**0.08**
WP	0.8	12.2	19.3	9.1	33.2	29.0	1.2	18.5	0.5	2.5	19.8	29.9	0.3	12.3	39.0	34.4	18.2	36.7	0.2	1.1	0.2	--

Figure 1. Overview of tetrasomic versus disomic expected transmission frequencies for **A)** single allele copies, **B)** two allele copies, and **C)** three allele copies based on possible gamete combinations for each scenario.

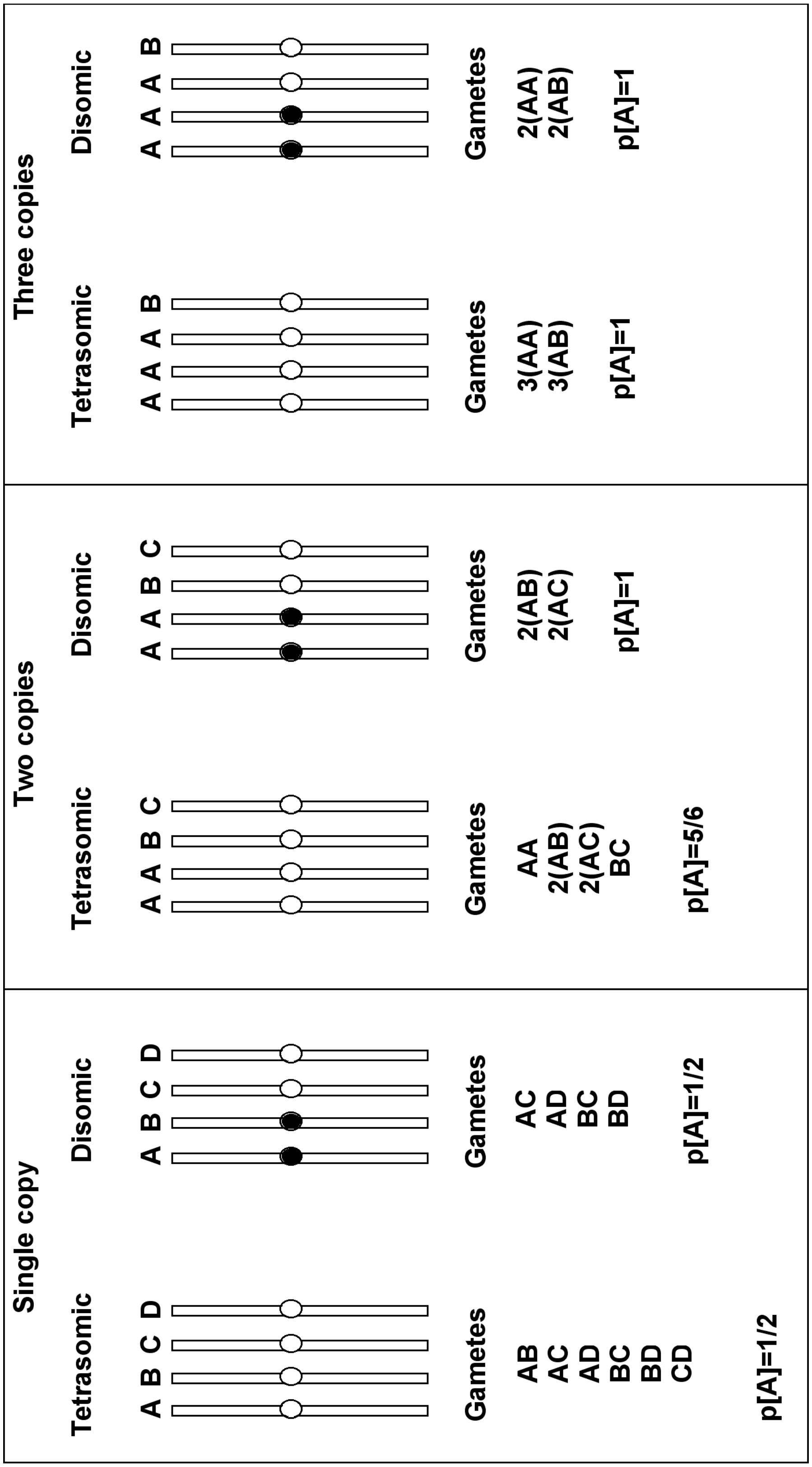

Single copy
Tetrasomic
A B C D
Gametes
AB
AC
AD
BC
BD
CD
p[A]=1/2
Disomic
A B C D
Gametes
AC
AD
BC
BD
p[A]=1/2
Two copies
Tetrasomic
A A B C
Gametes
AA
2(AB)
2(AC)
BC
p[A]=5/6
Disomic
A A B C
Gametes
2(AB)
2(AC)
p[A]=1
Three copies
Tetrasomic
A A A B
Gametes
3(AA)
3(AB)
p[A]=1
Disomic
A A A B
Gametes
2(AA)
2(AB)
p[A]=1

Figure 2. Illustration of double reduction following crossing-over during meiosis in tetraploids.

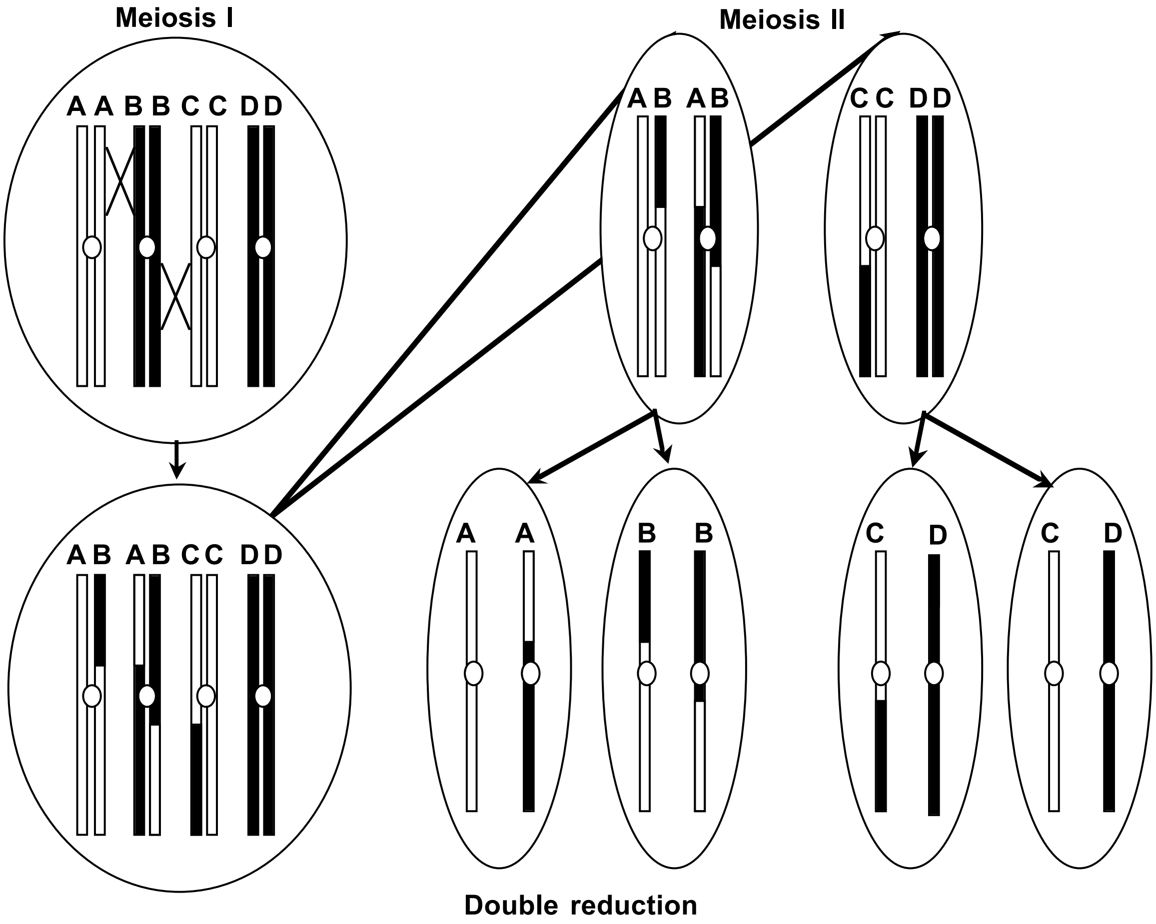
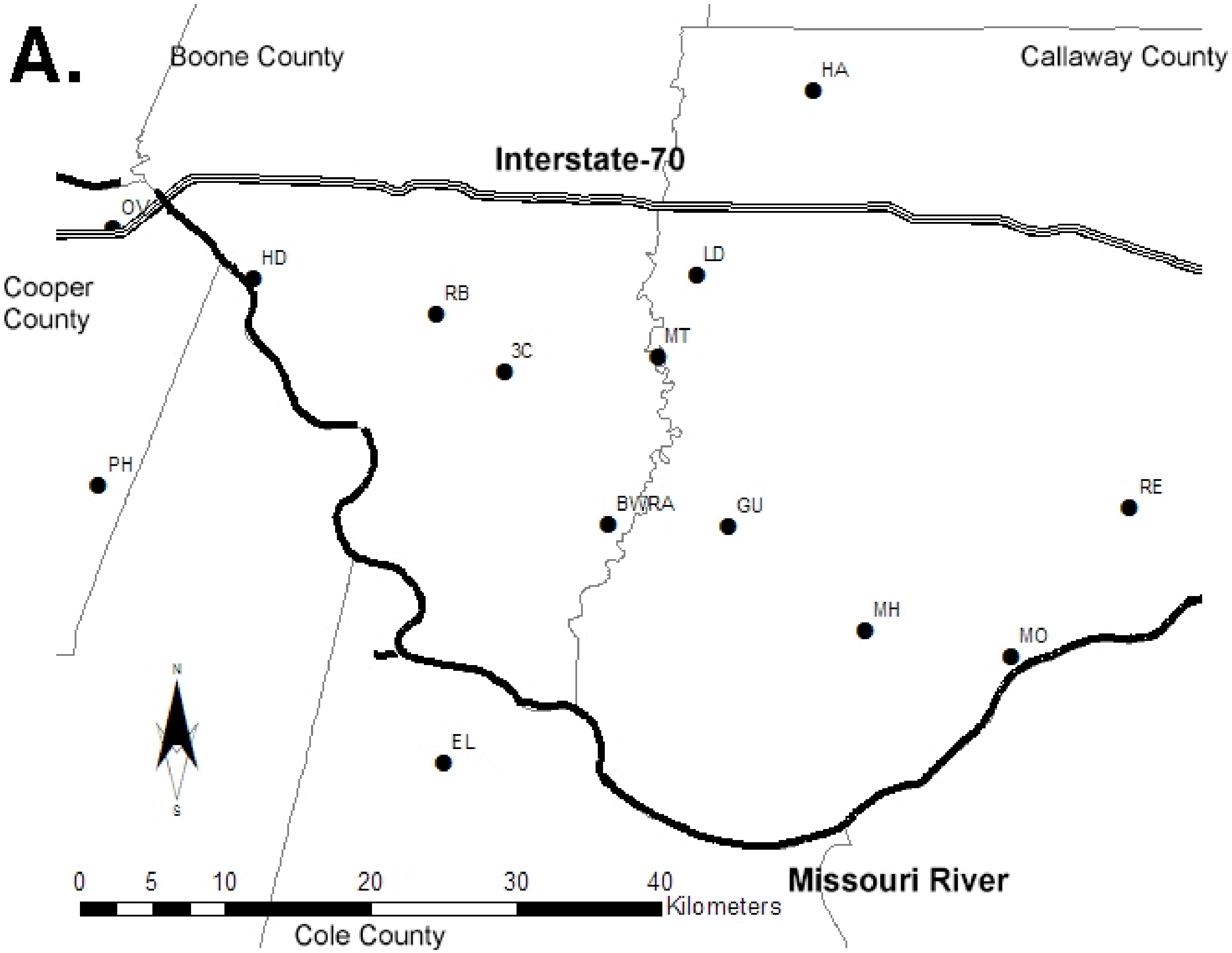

Meiosis I
A A B B C C D D
A B A B C C D D
Meiosis II
A B A B
C C D D
A A
B B
C D
C D
Double reduction

Figure 3. A) Diagram of transect sampling-scheme illustrating concentric circles of 10, 20, 30, and 40 km radii centered on **B)** the Baskett Wildlife Research Area (BWRA).


A.
Boone County
Callaway County
Interstate-70
Cooper County
OV
HD
RB
3C
LD
MT
HA
PH
BWRA
GU
RE
MH
MO
EL
N
S
0
5
10
20
30
40
Kilometers
Missouri River
Cole County

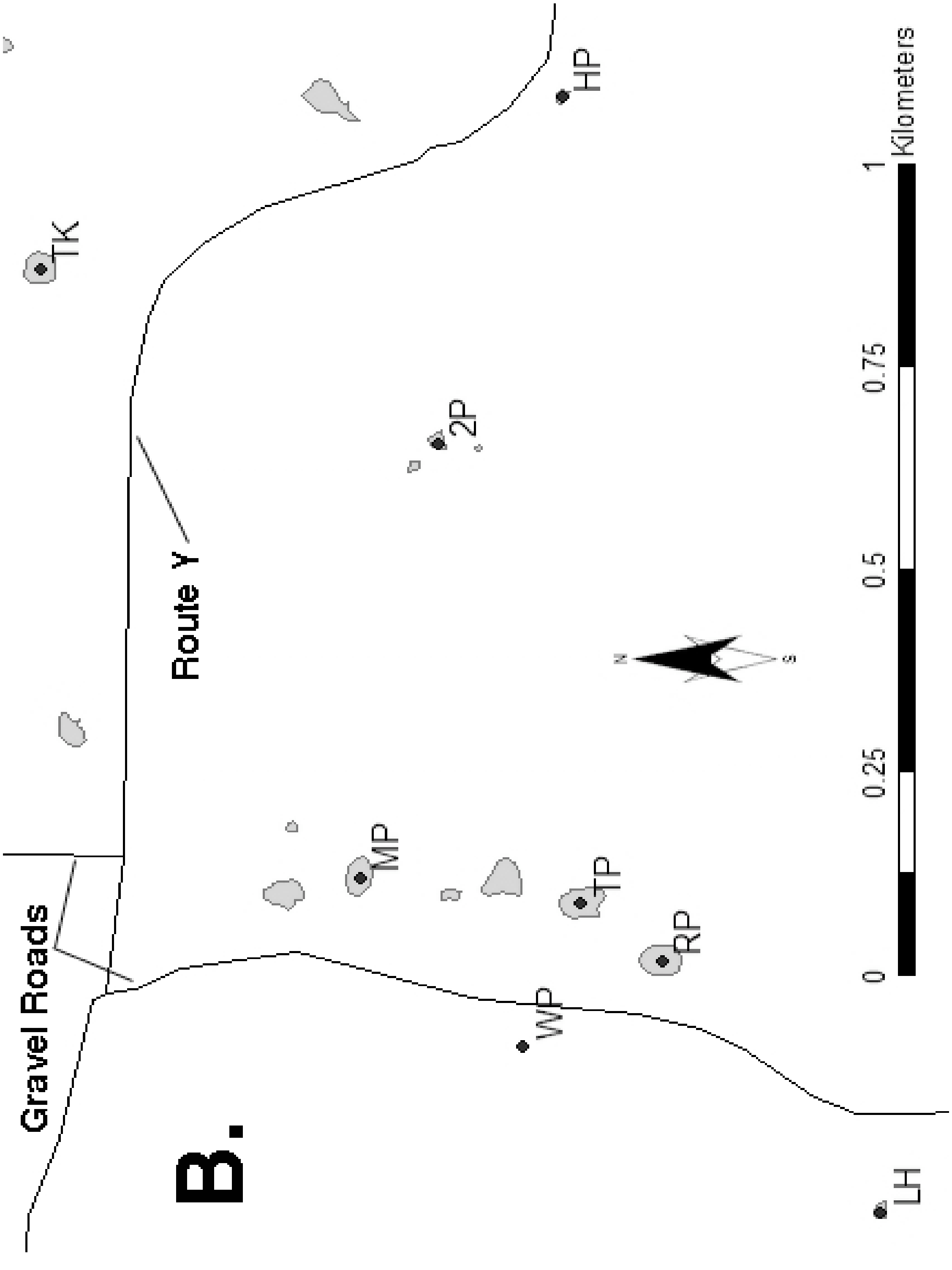

B.
Gravel Roads
Route Y
TK
HP
2P
MP
TP
RP
WP
LH
N
S
0
0.25
0.5
0.75
1
Kilometers

Figure 4. Correlation of genetic distance (pairwise F_{st}) and geographic distance with a trendline representing the relationship over all three microsatellite loci.

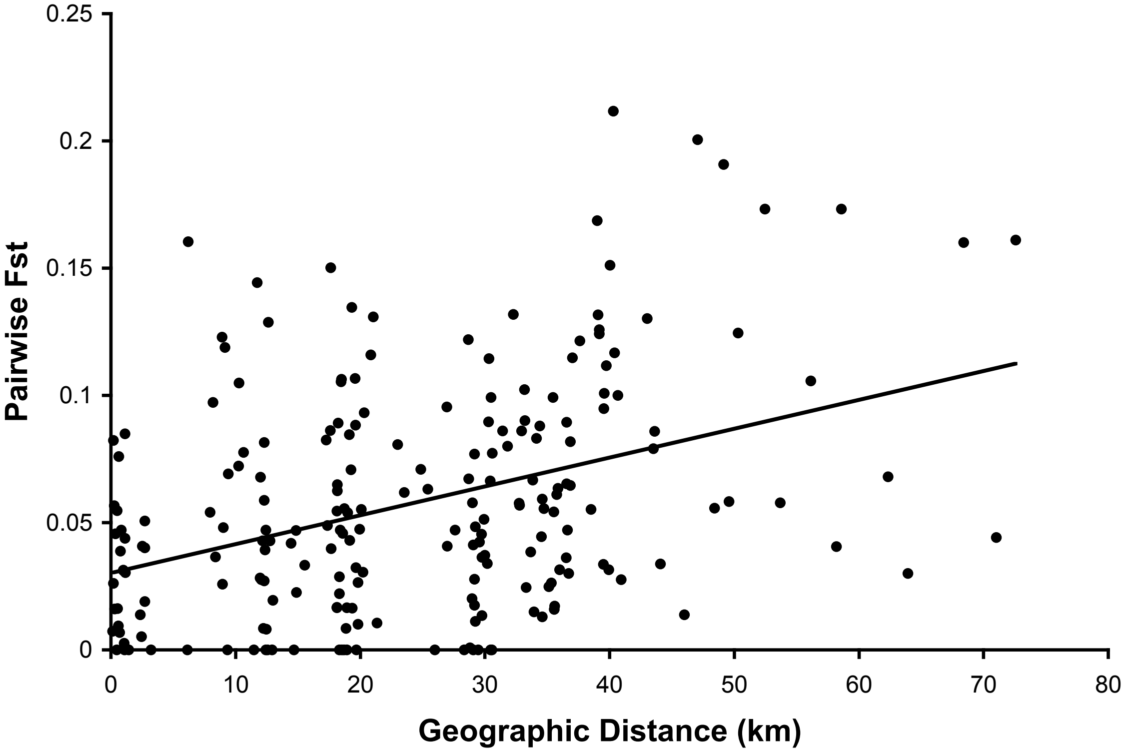
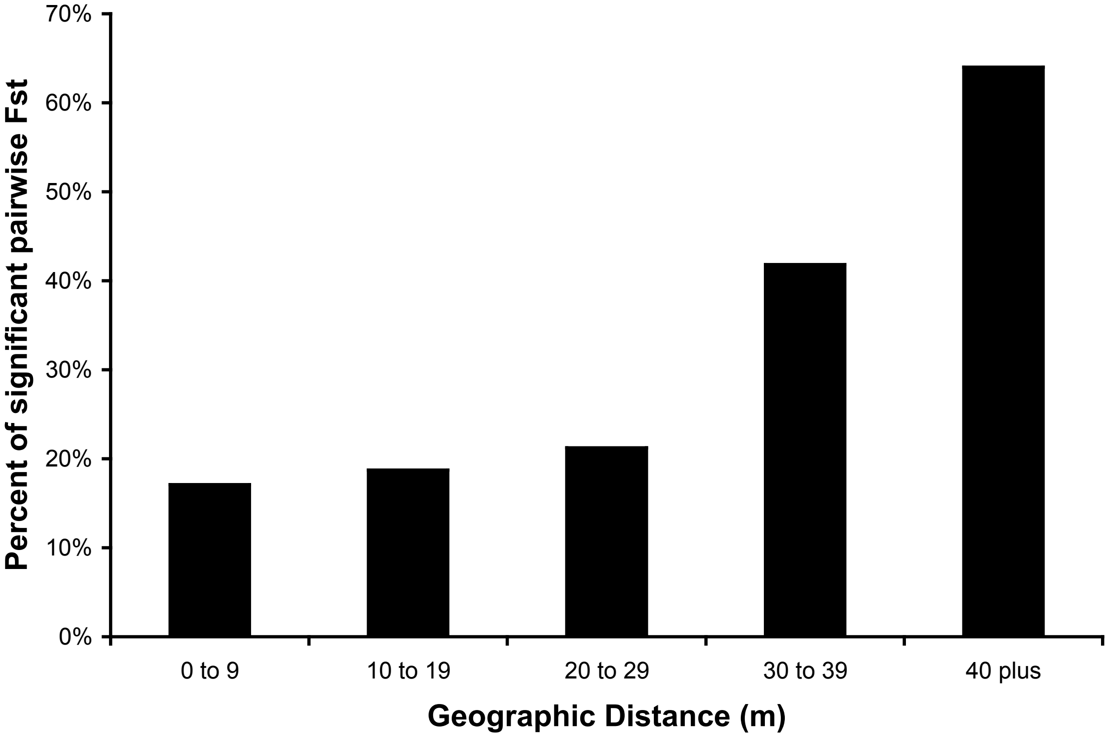

0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
40
50
60
70
80
Pairwise Fst
Geographic Distance (km)

Figure 5. Percentage of significant pairwise Fst values for five geographic distance categories.


70%
60%
50%
40%
30%
20%
10%
0%
Percent of significant pairwise Fst
0 to 9
10 to 19
20 to 29
30 to 39
40 plus
Geographic Distance (m)

Chapter 5

SUMMARY AND CONSERVATION IMPLICATIONS

Jarrett R. Johnson

BREEDING SEASON CORE HABITAT

1. Using artificial wading pools placed along transects extending from natural breeding ponds (Chapter 1), we determined the extent of within breeding season movements beyond natural pond boundaries that resulted in reproduction. Additionally, monitoring of artificial refugia (Appendix A; Chapter2A) during the breeding season generated data regarding the breeding season movements of individuals for non-breeding purposes.
2. We found that the majority of all eggs were deposited in pools within 15 m of natural pond edges. We suggested that a core habitat area extending at least 60 m from pond edges would encompass the extent of reproductive movements within single breeding seasons. Artificial refugia capture data demonstrated that the distribution of eggs deposited in wading pools more closely resembled the distribution of male individuals during the breeding season than the distribution of females.
3. With respect to conservation, our oviposition data (Chapter 1) illustrate that the creation of new ponds in proximity to existing ponds can be quickly colonized

even within a single breeding season and may therefore provide an immediate alternative in the case of stochastic negative changes to an individual's typical breeding location. Artificial refugia capture data (Chapter 2A) show that new ponds may be found during breeding season foraging activity by males and the likelihood of colonization of new sites may depend on the distribution of males adjacent to existing breeding locations. Capture data also indicate that loss of habitat immediately adjacent to breeding sites could hinder the foraging activities of males and negatively affect breeding choruses. From a metapopulation biology perspective, movement between breeding ponds within a single breeding season demonstrates that the 'pond as patches' view of amphibian metapopulations is inadequate. Our data support the concept that amphibian breeding habitat may be comprised of multiple ponds that experience frequent movements of individuals among them.

NON-BREEDING SEASON CORE HABITAT

1. I designed artificial arboreal refugia to capture treefrogs while absent from breeding sites (Appendix A), and placed the artificial refugia along transects extending from breeding sites (Chapter 2A). Monitoring of artificial refugia during the non-breeding season illustrated the distribution of each sex, and revealed differences between the distribution of individuals during both the breeding and non-breeding seasons. Tracking of individuals implanted with radiotransmitters (Appendix B, Chapter 2A) elucidated the extent of treefrog

migrations, and gut content analyses provided insight regarding the benefit of foraging at distances from breeding sites (Appendix C).

2. Our study showed that the distribution of males and females were significantly different and that the average location of females was more distant from breeding sites than the location of males. The breeding season and non-breeding season capture distributions of females were similar, however the distribution of males was constricted towards breeding ponds during the breeding season compared with the non-breeding season. Stomach content mass was significantly greater for animals captured in refugia than those captured at breeding ponds, and increased with distance from breeding sites for females but not for males. Radiotelemetry data revealed that migratory movements to overwintering sites extended at least 330 m into the terrestrial habitat adjacent to breeding sites.
3. These results provide further evidence that the ‘ponds as patches’ view of amphibian metapopulations is inadequate. Radiotelemetry data (Chapter 2A) indicate that the maximum extent of pond-breeding amphibian habitat patches can extend great distances beyond the boundary of breeding ponds. And artificial refugia data (Chapter 2A) illustrate that core habitat estimates can be more informative when they include information regarding the distribution of individuals in addition to the ‘maximum’ extent of the population. From a conservation standpoint, predictions regarding the effects of habitat loss require information regarding the relative distributions of individuals. In our study, elimination of the 200 m monitoring interval would result in a 50% loss of females and only a 13% loss of males. The importance of habitat distant to

breeding sites is supported by the stomach content data (Appendix C), which indicate that better foraging sites may be reached via longer distance migrations.

CORE HABITAT ASPECTS

1. Over the course of monitoring artificial refugia for treefrog captures (Chapter 2A) we recorded data regarding biotic and abiotic factors that could influence capture rates (Chapter 2B). Abiotic variables monitored included rainfall, temperature, and humidity, and the biotic variables investigated included tree species and tree diameter. Using radiotracking relocation points (Chapter 2B), we determined the effect of weather variables on microhabitat locations such as canopy, refuge, leaf litter, and soil. Furthermore, we investigated the relationship of gut content mass and the aspects of trees in which artificial refugia were located (Appendix C).
2. Our data illustrated that treefrog use of arboreal refugia was positively correlated with temperature and negatively correlated with humidity and rainfall. We also found that larger trees were used more frequently than smaller trees and that the species of tree had an effect on the number of captures recorded in each artificial refuge. Individuals were tracked to the forest canopy when rainfall values were high, to refugia when temperatures were high, to the leaves when both temperature and rainfall were low. The presence of individuals in or under the soil was most highly positively correlated with the date. The stomach content masses of males were positively associated with tree diameter, but no relationship was found for females. We found an effect of tree species on the mass of stomach

contents, with animals captured in refugia attached to white oak trees containing the greatest masses of prey items.

3. The implication of these investigations is that knowledge of the important terrestrial habitat features used by amphibians allows for more effective conservation planning. We have shown that microhabitat use varies depending on both biotic and abiotic factors, which suggests that effective management of amphibian populations depends on providing access to an array of microhabitat locations. Maintaining adequate tree cavity densities and appropriate leaf litter layers are important to sustaining treefrog populations. From an applied standpoint, selective harvesting of large trees may negatively affect the foraging behavior of treefrogs, as would the removal of white oak trees. The timing of forestry activities may also differentially affect treefrog populations depending on microhabitat use.

INTERPOND DISTANCE AND MATRIX HABITAT

1. We constructed an experimental landscape of cattle tank ponds separated by two treatment distances across two treatment matrix habitat types (Chapter 3), to discover the effects of interpond distance and intervening habitat on the frequency of dispersal among breeding locations. We used both mark-release/recapture and genetic techniques to identify individuals, and recorded movements of both juveniles and adults.
2. This study determined that movements through field habitat were twice as infrequent as movements through forested habitat, and that the effect on juveniles

was increased to a three fold reduction in movements across field habitat. Movements of 180 m were equally as frequent as movements of 60 m except for adult movements within breeding seasons, when 60 m movements were twice as frequent as 180 m movements. Most importantly, we found an interaction between matrix habitat type and inter-pond distance. Movements across 60 m distance treatments occurred with equal frequency in each habitat type, but movements through 180 m forest treatments occurred four times more frequently than movements through 180 m field treatments.

3. These data suggest that fragmentation of forested habitat can severely limit the frequency of dispersal movements across a landscape. According to metapopulation theory, connections among populations are crucial to decreasing regional extinction probabilities. While local extinctions temporarily leave some patches empty, recolonization via dispersal from adjacent sites allows the metapopulation to persist. Interruption of natural movements among populations disrupts the extinction/recolonization balance and local extinctions become more permanent. Landscape level conservation planning for amphibian populations must consider the interaction of distance and matrix habitat in maintaining connections among populations in order to make effective predictions regarding extinction probabilities and regional persistence.

PATTERNS OF GENE FLOW

1. I used microsatellite loci to elucidate fine-scale patterns of gene flow among natural breeding sites (Chapter 4). Pairwise estimates of genetic divergence were

correlated with measurements of geographic distance to determine the scale at which genetic differentiation among breeding sites occurred and therefore infer the scale of dispersal movements. Estimates of genetic differentiation and dispersal distances provided information regarding the scales at which classical metapopulation dynamics and 'patchy' population dynamics were most prominent.

2. I found that significant differentiation among breeding ponds occurred even over very short geographic distances. Overall however, sites had a moderate probability of differentiation if separated by less than 30 km. Sites separated by more than 30 km experienced the highest probability of genetic differentiation.
3. The results demonstrate that significant differentiation among sites can occur even when the movement of individuals between sites is high, perhaps do to the extreme variation in yearly amphibian population sizes. More importantly, this study illustrated that metapopulation dynamics are most likely important when populations are separated by up to 30 km, and that 'patchy' population dynamics should prevail when population were separated by only a few kilometers. Conservation biologists can use this information to make decisions regarding the potential of adjacent sites to exchange individuals. Populations isolated by 30 km will most likely not be naturally recolonized following local extinction and priority should be given to populations that have a greater probability of long-term persistence.

Appendix A

A NOVEL ARBOREAL PIPE-TRAP DESIGNED TO CAPTURE THE GRAY TREEFROG (*HYLA VERSICOLOR*)

Jarrett R. Johnson

INTRODUCTION

Drift fences with pitfall traps are a common and effective method of censusing amphibian populations (Dole 1971; Gibbons and Bennett 1974; Gibbons and Semlitsch 1982; Shoop 1965). But some studies have noted that drift fence arrays are inappropriate for capturing hylid frogs because of their ability to climb vertical surfaces (Dodd 1991; Gibbons and Bennett 1974). The difficulty of capturing treefrogs using drift fences has led to the development of techniques for sampling populations of hylid frogs including polyvinyl chloride (PVC) pipes, elevated inverted tin cans, and modified drift fences (Boughton et al. 2000; Goin and Goin 1957; Moulton et al. 1996; Murphy 1993). Currently, the most widely used of these methods consists of constructing artificial PVC pipe-trap refugia. Several studies have found that varying pipe-trap designs and configurations lead to differences in capture success of species and size classes (Boughton et al. 2000, Moulton et al. 1996, Bartareau 2004), suggesting that no single pipe-trap design is appropriate for all hylid species in all locations. Here I report on the effectiveness of a novel artificial pipe-trap designed to simulate natural arboreal retreats and capture the gray treefrog (*Hyla versicolor*) in central Missouri.

METHODS

The pipe-traps used in this study consist of 60 cm long sections of 3.8 cm inside diameter black acrylonitrile butadiene styrene (ABS) pipe (Fig. 1). A 10 cm upper and 15 cm lower section of 5.0 cm inside diameter black ABS pipe are attached with wire to bungee cords tied around trees (Fig. 2) to allow the smaller diameter pipe to slide freely up and down during monitoring (Fig. 3). The flexibility of the bungee cord allows the 5.0 cm diameter sections of pipe to twist during monitoring, but remain at the correct position on the tree. Utility wire is used to tighten the grip of the bungee cord on the upper and lower sections of the trap (Fig. 2) and to provide a pivot point during monitoring (Fig. 3B). Traps are sealed at the bottom with 5.0 cm diameter black ABS caps to allow for rainwater to fill the lower sheath of 5.0 cm diameter pipe (Fig 1.). Water levels remain constant because the inside pipe sits loosely within the bottom capped portion, and excess rainwater drains out. Frogs may enter and leave the pipe-trap freely at the upper opening (Fig. 1).

The pipe-trap design outlined here is similar to some PVC pipe designs evaluated by Boughton et al. (2000). However, I modified their designs in three ways. First, black ABS pipe was used rather than white PVC pipe to provide more realistic (i.e. dark) refugia sites (Lohoefener and Wolfe 1984), but other possible advantages may include warmer internal temperatures and greater camouflage from potential predators. ABS pipe is typically used for residential plumbing applications and is similar in function to the polyvinyl chloride (PVC) piping used in previous studies of artificial arboreal refuge use.

Second, I eliminated the need to pound nails directly into trees. Avoiding damage to trees can be an important consideration in old growth forests, or on public lands that discourage the permanent disturbance of habitat. Lastly, this pipe-trap design maintains a constant water depth within the pipe-trap. If frogs are attracted to pipe-traps because of the water they contain, it is important to minimize water-level differences among traps.

The pipe-trap refugia described in this report were constructed for an ongoing study of gray treefrog dispersal distances and terrestrial habitat requirements. To date, pipe-traps have been monitored during two fall emigrations as frogs move towards overwintering sites, two spring immigrations as frogs move towards breeding sites, and two summer breeding seasons (Table 1). Here I report data regarding the effectiveness of these traps for capturing gray treefrogs in Midwestern U.S. deciduous forests throughout the breeding and non-breeding seasons.

A preliminary monitoring period was undertaken during the fall of 2002 and the spring of 2003 (Table 1) at two gray treefrog breeding sites located within the Thomas Baskett Wildlife Research Area near Ashland, Boone County, Missouri. I placed 28 pipe-traps with the upper opening 3 m above ground onto large diameter deciduous trees and arranged them at fixed distances from breeding ponds along four transects. Each transect extended from the breeding sites into secondary growth (~100 yr) oak/hickory (*Quercus spp.*/*Carya spp.)* forest with sugar maple (*Acer saccharum*) understory up to a distance of 200 m. I expanded the number of pipe-traps to 100 during the summer 2003 monitoring session (Table 1). Existing transects received an extra pipe at each distance, and three additional paired transects were added to a third gray treefrog breeding site, for a total of seven paired transects extending from three breeding ponds into the adjacent forest. During monitoring, pipe-traps were checked during daylight hours for the presence of treefrogs. If present, frogs were extracted from the pipes by forcing a sponge through one end until the frog could be reached at the opposite end (Fig. 3C), as described by Boughton et al. (2000). Individuals were then given unique toe-clip identifications and snout-vent length was measured with a plastic ruler. After processing, frogs were returned to the pipes and placed back on the tree.

RESULTS

In total, pipe-traps captured 137 treefrogs (67 males, 43 females, and 28 juveniles) a total of 759 times (Table 2). The range of snout-vent lengths (SVL) was 17-56 mm, which encompasses the entire range of adult gray treefrog lengths (32 to 51 mm) as described by Conant and Collins (1998). The diversity of lengths recorded in this study indicates that these pipes are appropriate for capturing all size classes of *Hyla versicolor*. However, without estimates of actual treefrog abundances at each size class, it remains unclear if these data display a bias in capture rates generated by the internal pipe diameter as suggested by Martin et al. (2003) and Bartareau (2004). Multiple frogs were recorded in these pipes much less frequently than reported by Boughton et al. (2000) and Moulton et al. (1996), and occurred only on 20 occasions (3%). Only once were three frogs captured in the same pipe. On 7 occasions (1%) frogs near the opening of the pipe escaped up the tree before they could be captured, and infrequently frogs that were replaced in the pipes following processing exited the pipe, as has been reported by Boughton et al. (2000).

Other vertebrate species captured in the traps include one adult spring peeper (*Pseudacris crucifer*), one adult five-lined skink (*Eumeces faciatus*), ten mice (*Peromyscus spp.*), and six flying squirrels (*Glaucomys volans*). I observed no amphibian mortality within the pipes, however 13 of the 16 mammals encountered were dead. Prior studies using pipe-trap refugia for monitoring amphibians have not reported mammalian mortality. Use of this pipe-trap design may not be appropriate for use in areas in which such mortality is of great concern. Modification of the open end of the pipe-trap to reduce the frequency of mammalian intruders while not affecting treefrog access may be necessary. I periodically replaced the water in the traps to eliminate organic debris (e.g. invertebrates, plant material) that had accumulated at the bottom of the pipes.

DISCUSSION

Previous studies using PVC pipe refugia have been conducted primarily in the southeast U.S. to monitor other species of hylids (*H. avivoca, H. cinerea, H. femoralis, H. gratiosa*). Artificial refugia studies in the southeast have been successful in capturing treefrogs in pipe-traps inserted upright into the ground, placed open-ended or capped at varying heights on trees, and in 'T' configurations (Bartareau 2004; Boughton et al. 2000; Moulton et al. 1996). However, of the few attempts that have been made to implement similar pipe-trap designs in more northerly locations (MI, Ball 1996; NY, Tregger 2004) none have succeeded in capturing large numbers of treefrogs. When compared to the number of captures reported in this study, the lack of gray treefrog captures in Michigan and New York using open-ended ground mounted pipes and bottom-capped pipes hanging from branches respectively, indicate that this new pipe-trap design may be more appropriate than previous designs for sampling northern populations of hylids. Furthermore, these data suggest that use of pipe refugia need not be limited to the southeast U.S., and specifically can be used to capture gray treefrogs.

Additional data regarding the factors that drive the use of artificial refugia would be beneficial to the development of pipe-traps designed to optimize captures of particular species in specific locations. While the use of pipe-trap refugia to generate estimates of treefrog density or abundance may be complicated by biases of pipe diameter and design configuration, artificial pipe-trap refugia may be especially useful for delineation of terrestrial core habitat used by hylid frogs during both the breeding and non-breeding seasons when traditional methods of capturing amphibians are inadequate.

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Table 1. Summary of monitoring sessions and transect arrangement for each season.

Season	# of pipe-traps	Monitoring interval (d)	Duration	# of monitoring days
Fall 2002	28	5	30 Sept. to 15 Oct.	4
Spring 2003	28	2	17 April to 05 May	10
Summer 2003	100	2	17 June to 08 July	11
Fall 2003	100	2, 3	11 July to 23 Aug., to 22 Oct.	42
Spring 2004	100	2	09 April to 14 May	18
Summer 2004	100	2	18 May to 11 July	28
Fall 2004	100	2	13 July to 24 Oct.	52
Spring 2005	100	3	05 April to 13 April	4

Table 2. Summary of the number and type of captures recorded during each monitoring session.

Season	Total captures	# of recaptures	# of males	# of females	# of juveniles
Fall 2002	7	2	3	1	1
Spring 2003	20	12	4	1	3
Summer 2003	20	5	3	10	2
Fall 2003	290	222	31	16	21
Spring 2004	83	38	18	13	14
Summer 2004	145	91	29	17	8
Fall 2004	177	132	18	16	11
Spring 2005	30	4	8	9	9

Figure 1. Pipe-trap design. The smaller diameter inside pipe slides freely through the larger diameter top sheath and rests inside the capped bottom portion of large diameter pipe. The gap between the small and large diameter pipe maintains water level at a constant depth.

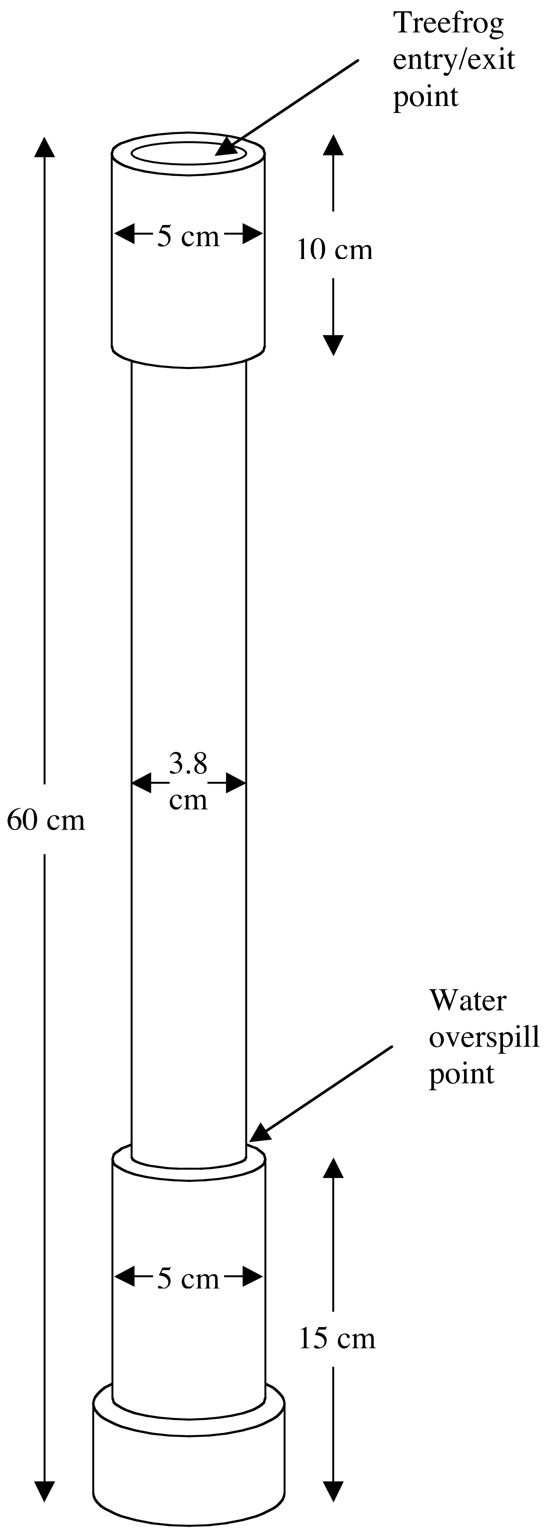

Treefrog entry/exit point
10 cm
5 cm
3.8 cm
60 cm
Water overspill point
5 cm
15 cm

Figure 2. Top view of a pipe-trap attached to a tree. The bungee cord and utility wire attachment is repeated on bottom segment of 5 cm D pipe.

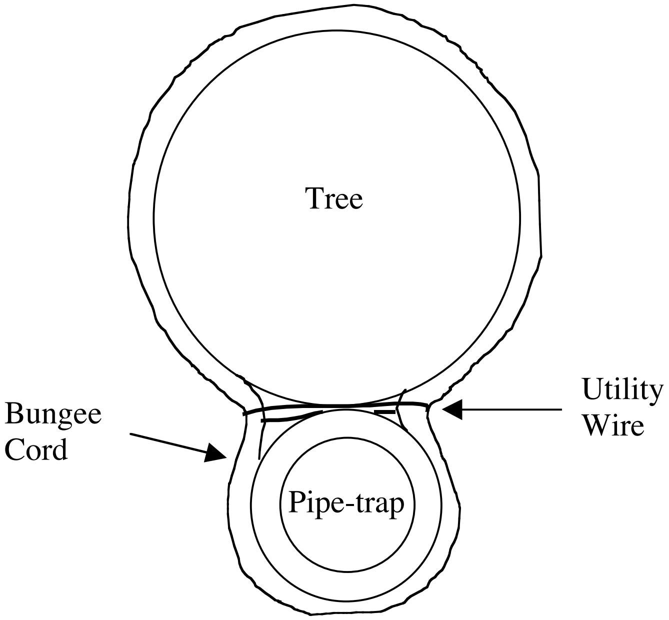
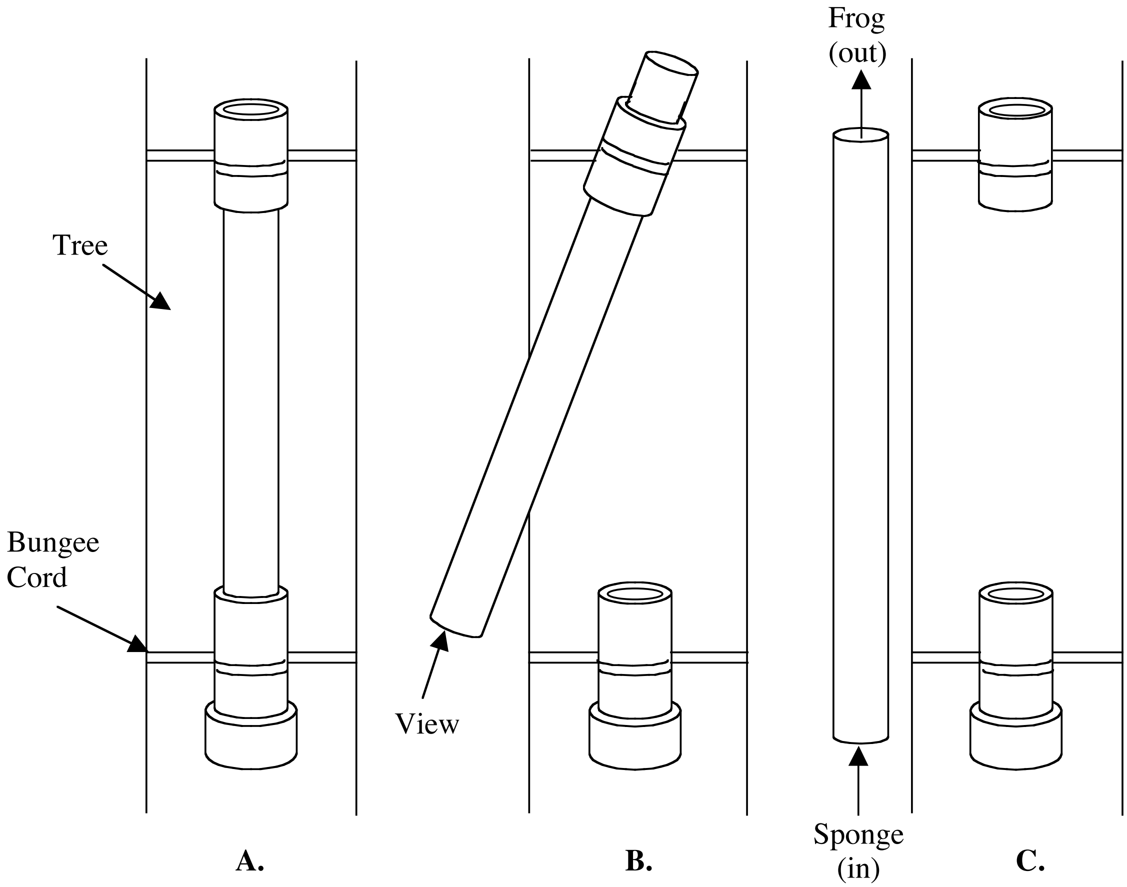

Tree
Bungee Cord
Utility Wire
Pipe-trap

Figure 3. Overview of pipe-trap monitoring procedure. **A.** Normal pipe-trap position. **B.** Inside pipe is slid upwards and top sheath twists on bungee cord so the observer can detect frogs from below. **C.** If a frog is present, the inside pipe is removed and the frog is extracted.


Frog
(out)
Tree
Bungee
Cord
View
Sponge
(in)
A.
B.
C.

Appendix B

SUCCESS OF INTRACEOLOMIC RADIOTRANSMITTER IMPLANTATION IN THE TREEFROG, *HYLA VERSICOLOR*

Jarrett R. Johnson

INTRODUCTION

Concern over the status of amphibian populations across the globe has led to an increase in the need for information regarding the location and habits of individuals throughout their life cycle. Determination of terrestrial habitat requirements (i.e. space-use estimates) and quantification of migration and dispersal distances are crucial pieces of information when the goal is determining the prospects of population persistence in the face of increasing habitat modification. These data are particularly difficult to obtain for many amphibians due to their cryptic lifestyle when away from breeding sites. Although amphibians may be easily located and studied during the breeding season when they are congregated in large numbers, the majority of their time is spent in the terrestrial habitat where they are typically secretive and difficult to find. Many methods of tracking amphibians as they emigrate from breeding aggregations have been developed (e.g. thread trailing, radioactive tagging, fluorescent powders), but the most successful method of elucidating amphibian activities at distances from breeding sites has been radiotelemetry.

For amphibians, radiotelemetry requires that individuals have transmitters implanted intracoelomically (Sinsch 1988, Smits 1984, Stouffer et al. 1983), or subcutaneously (Werner 1991), attached externally via backpacks (VanNuland and Claus 1981, Kingsmill 1991) or waistbands (Fukuyama 1988, Rathbun and Murphy 1993), or contained within the gastric cavity following forced ingestion (Oldham and Swan 1991).

The most appropriate method of attachment depends on the morphology of the target species and the particular habitat in which tracking will occur. For a study of the nonbreeding-season movement and activity patterns of the gray treefrog (*Hyla versicolor*), frogs were surgically implanted with radiotransmitters into the coelomic cavity. Implantation was preferable due to concern that backpacks would hinder the ability to maneuver in tight spaces (e.g. treeholes) and waistbands would be easily escapable for the slender treefrogs. Although ingestion of transmitters has been shown not to influence feeding behavior on larger amphibian species (Oldham and Swan 1991) or snakes (Plummer and Congdon 1994), I was hesitant to use forced ingestion for gray treefrogs given the relative size of the transmitter.

Implantation of foreign elements into the body cavity has been reported to have pathological and behavioral effects on birds (Mulcahy and Esler 1999) and mammals (Guynn et al. 1987), but limited information exists regarding the effects of implantation in amphibians. In fact, limited procedural techniques have been reported for insertion of transmitters (or dataloggers) into the body cavity of amphibians (see Bradford 1984, Madison 1997, Sinsch 1988, Smits 1984, Stouffer et al. 1983) despite the preference of the method for the relocation of marked animals in a variety of habitats. While general surgical techniques for amphibians can be found in Wright (2000, 2001a, and 2001b), this report details the surgical procedures used for implantation and subsequent removal of transmitters in gray treefrogs, and provides insight into the fate of individuals during the weeks following the implantation surgery and one-year post a second survival (i.e. removal) surgery.

METHODS

Animals

Twenty-two adult (10 male and 12 female) gray treefrogs were captured in experimental arboreal retreats constructed out of ABS (Acrylonitrile Butadiene Styrene) conduit during the course of a separate study (Chapter 2). Frogs were selected based on timing of capture (approx. 25 d prior to 1st 0^{o} C night) and body mass. Transmitters were not to exceed 10% of an individual's mass (Richards et al. 1994), and only frogs weighing greater than 8.5 g were included in the study. At the time of capture, the average length of individuals was 50.8 mm (±1.2 mm SE) snout to vent (SVL) length and the average weight was 11.5 g (±0.9 g SE). Individuals were kept overnight in individual ventilated plastic containers (23.5 x 15.2 x 16.5 cm) with several wetted paper towels until surgery the following day.

Within 24 hours following implantation, individuals were released at the point of capture and tracked for the duration of the transmitter battery life (~25 days). Before battery failure, radiotransmitters were removed and animals were allowed to recover in clean plastic containers with newly moistened paper towels for at least 24 hours before returning to the field. Prior to release, the behavior (i.e. body posture, avoidance behavior) of each animal had returned to normal. Monitoring of experimental arboreal refugia continued through the spring of 2005, and any animals recaptured post surgical procedures were recorded.

Radiotransmitters and surgical procedures

Twelve 0.85 g BD2 radiotransmitters (14 x 6.5 x 3.5 mm) with internal helical antennae were purchased from Holohil Systems Ltd. (Carp, Ontario, Canada K0A 1L0) and had a expected battery life of approximately 28 days in 2003 and 25 days in 2004. Transmitters and antennae were encapsulated in an inert waterproof epoxy and were soaked in ethyl alcohol and rinsed with sterile water immediately preceding implantation.

Ten frogs were captured for transmitter insertion surgery from 2 – 21 October 2003 and 12 frogs were captured from 27 September to 14 October 2004. All surgical procedures were performed in an approved animal care facility on the University of Missouri campus in Columbia Missouri, USA. Prior to surgery, a laboratory table was sterilized with a 10% bleach solution followed by ethyl alcohol. Surgeries were performed on new paper towels moistened with sterilized water, under a dissecting microscope with supplemental fiber optic light source. Latex examination gloves were worn throughout the surgical procedures.

Prior to surgery each individual was weighed with a digital scale and measured with a digital caliper, and submerged in a 0.2% (in 2003) or 0.4% (in 2004) aqueous solution of ethyl-*m*-aminobenzoate methanosulfonate (MS-222, Sigma-Aldrich Co., St. Louis Missouri 63103 USA) buffered to pH ~7 with sodium bicarbonate (NaHCO3, Wright 2001a). MS-222 is commonly used to anesthetize fishes and amphibians (see

Downes (1995) for a discussion of appropriate concentrations). Frogs were held in the anesthetic solution until loss of righting behavior and lack of response to toe pinching ($\bar{x}$=6 min, ±2.5 min SE) indicated deep sedation. Immediately following anesthesia, frogs were rinsed with sterile water to remove residual MS-222 (Wright 2001), although other reports of amphibian surgery suggest that individuals be held in the anesthetic solution for the duration of the procedure to maintain hydration and unconsciousness. No breathing or buccopharyngeal movement could be detected from anesthetized frogs but the heartbeat remained visible under the dissecting microscope when dorsally recumbent.

Following removal from the MS-222 solution, frogs remained immobile for just under 30 minutes. Obvious breathing and gulping behavior preceded awakening. On occasion, supplemental drops of anesthesia solution were administered to the throat and chest region to maintain sedation and prevent desiccation. Individuals righted themselves approximately 20 minutes post surgery.

Radiotransmitter implantation consisted of a 6 mm incision in the ventrolateral abdominal musculature lateral from and parallel to the ventral midline and anterior to the right hind limb (Figure 1). The position of incision was chosen to avoid major veins and minimize rubbing of the wound by the hind limbs and substrate. Transmitters were inserted into the coelomic cavity and positioned parallel to the intestines and gonads (Stauffer et al. 1983). Between 5-6 sutures were used to close the musculature and the epidermis separately (Wright 2000) with absorbable 4–0 (in 2003) or 5–0 (in 2004) chromic gut monofilament (Ethicon Inc. Somerville, New Jersey 08876 USA).

Bennett (2000) reports that chromic gut sutures are a source of irritation in several taxa, including reptiles, but gives no mention of any effect on amphibians. I did not observe any irritation that could be attributed to the sutures. Wright (2001b) suggests using non-absorbable monofilament sutures for the epidermis, as absorbable sutures have been reported to dissolve too rapidly in moist amphibian habitats. My results support this contention when using 5-0 sutures, as surgeries performed in 2004 had a higher occurrence of dehiscence than in 2003, but 4-0 sutures appeared to be sufficiently large to persist until wounds had healed. The arboreal habits of adult gray treefrogs may allow absorbable sutures to persist for an appropriate duration for wound healing. Furthermore, incision sites were sealed with VetBond™ (3M Animal Care Products St. Paul, Minnesota 55144 USA) tissue adhesive, which provided additional strength to the sutures. Proper dosages of analgesics for amphibians have been poorly documented (Wright 2001b), so the topical analgesic Lidocaine (2%; Alkorn Inc. Buffalo Grove, Illinois 60089 USA) was used sparingly, and consisted of a small amount applied to the incision site after VetBond™ was dry and the frog regained consciousness. Temporary redness from Lidocaine was observed in some instances.

Extraction of radiotransmitters was accomplished with sterilized forceps (Bennett 2000) via a slightly larger incision (7 mm) in a similarly lateral position on the opposite side from the initial surgical site. Extrication incisions were closed with 6-7 stitches in the muscle and between 7-8 stitches in the epidermis, followed by VetBond™ tissue adhesive on the surface of the wound. Frogs were allowed to recover from their second surgery in individual plastic containers with moistened paper towels for several days (depending on the outside temperature) before being permanently released.

RESULTS

Seven of the 22 frogs containing radiotransmitters were lost during the course of the two study periods (2003 & 2004) or not recovered following termination of the study. At least one disappearance was from a predation event and the rest were most likely due to battery failure before recapture for transmitter extraction. Some wounds healed more rapidly than others, however all insertion wounds healed before extraction procedures were performed. All sutures in muscle tissue maintained their integrity, however early surgeries with fewer epidermal sutures (2-3) did show some evidence of minor dehiscence of the skin incision. Upon recapture, these frogs appeared to remain healthy and free from infection.

Three of the five individuals lost due to presumed battery failure (frogs #1026, #4032, and #4070) were recaptured in arboreal retreats approximately six months following implantation. A large amount of fibrous tissue had accumulated around the surface of the transmitter implanted in #1026 than those that had been removed after the typical 25-day period, and that individual expired several days following removal surgery. Excess tissue was not observed in individuals #4032 and #4070, and the extraction surgeries were successful. No other direct mortality was observed from implantation procedures. There was no evidence of infection at or around the incision sites, however individual #0895 developed swelling in the right hind limb during the course of tracking, and died several days following the transmitter removal surgery. Individuals weighed an average of 1.1 g (9%) less directly following the removal of the radiotransmitter than they did directly before implantation (Table 2).

Six of the seven frogs that underwent transmitter removal surgery in 2003 were recaptured during 2004 (Table 1). These individuals showed varying degrees of scarring that appeared to be a corollary of the investigator's improvement in surgical proficiency (Fig. 2). All six animals were recaptured in the same experimental arboreal refuge in which they were initially captured (before surgery) and appeared to be behaving normally. One female (#0769) was observed in amplexus at a breeding site, and traveled 100 m back to her diurnal refuge site within a 48 hr period. During brief monitoring in the spring of 2005 (5 April to 14 April), four individuals tracked in 2004 were also recaptured following overwintering (Table 1). There is a good chance that additional individuals from 2004 would have been detected with increased sampling duration.

DISCUSSION

Intraperitoneal implantation of radiotransmitters did not adversely affect gray treefrog behavior while implanted, or following removal. The vast majority of individuals were recaptured within their presumed non-breeding season foraging grounds between 120 and 543 days following their final surgeries. Similarly, frogs that were not subject to surgical procedures also were recaptured within their fall foraging sites during spring monitoring of artificial refugia (Chapter 2A). All recaptured surgery animals appeared to be in good health, were active, and did not display any external malformations as a result of two major survival surgeries.

While the procedures outlined subject these animals to stress, these data suggest that normal behavior resumes quite rapidly and long-term effects are minimal. Other studies that have used less invasive external transmitter attachment procedures (e.g. waistbands) have reported problems with external lesions on the skin after prolonged attachment of transmitters. Certainly, these irritations can pose a great stress to individuals due to the prolonged duration of the irritation, and as a result, bias any movement data collected. Similarly, individuals #1026, #4032, and #4070 demonstrate that the extended presence of a transmitter within the body cavity does not prevent successful overwintering, but may lead to complications during extraction. The duration of any study involving tracking of amphibians should be carefully considered given these limitations.

Most (if not all) of the animals used in this study were able to survive the winter directly following a second surgical procedure barring extraneous circumstances. These data suggest that the standard Animal Care and Use Committee protocol requiring that animals be euthanized instead of undergoing a second major survival surgery, be considered on a case-specific basis. The possible benefits to the return of individuals to a population (especially in the case of endangered species) may outweigh the temporary stressor of additional surgery in some instances.

The loss of weight during the duration of the study is concerning given that the primary activity of anurans between breeding and hibernation is to forage and restore energy reserves that were used during breeding activities. However, all animals were able to survive the winter and resume foraging the following spring and #0769 was healthy enough to produce a clutch of eggs during the breeding season following two major survival surgeries (Fig 2). Without information regarding typical patterns of weight loss (or gain) of amphibians following a prolonged breeding season, the observed weight loss is difficult to put into context. However, during a study of the effect of desiccation on body mass of gray treefrogs, individuals were able to recover from weight loss up to 30% and typically varied by 5% in the control group (unpub. data).

To my knowledge no other study has reported recaptures of radiotracked individuals following removal of internally housed transmitters. Surgeries of this sort have been performed in the past (Sinsch 1988, Stouffer et al. 1983, Seitz et al. 1992), but little information exists regarding the effects of surgical procedures following multiple major survival surgeries. I have found that multiple surgeries apparently do not adversely affect individuals, and when done properly, may be appropriate for use on critically imperiled species. When followed, the methods outlined in this study will help to generate crucial information regarding the movements and activities of moderate to large sized amphibians.

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Table 1. Surgery and last recapture dates for all animals tracked during 2003 and 2004 that underwent survival transmitter removal. All recaptures were recorded in experimental arboreal refugia.

Animal ID	Sex	Extraction surgery date	Initial recapture date	Number of days until 1st recapture	Final recapture date	Minimum days surviving
0330	M	7 Dec 2004	13 Apr 2005	127	13 Apr 2005	127
0736	F	19 Nov 2003	16 Apr 2004	149	22 May 2004	185
0745	M	31 Oct 2003	23 Apr 2004	175	13 May 2004	195
0769	F	2 Nov 2003	9 Apr 2004	159	10 Apr 2005	543
0837	M	3 Nov 2003	N/A	N/A	N/A	N/A
0881	F	6 Dec 2004	5 April 2005	120	5 April 2005	120
0884	F	18 Nov 2003	23 Apr 2004	157	19 Aug 2004	275
0888	M	30 Oct 2003	N/A	N/A	N/A	N/A
0895	F	5 Nov 2004	N/A	N/A	N/A	N/A
1026	M	3 Oct 2003*	23 Apr 2004	203	23 Apr 2004	203
1161	F	6 Dec 2004	N/A	N/A	N/A	N/A
1720	M	6 Dec 2004	N/A	N/A	N/A	N/A
2838	F	16 Nov 2003	21 Apr 2004	157	21 Apr 2004	157
4032	F	8 Oct 2004*	5 Apr 2005	179	5 April 2005	179
4070	F	8 Oct 2004*	13 Apr 2005	187	13 Apr 2005	187
4074	F	3 Nov 2004	N/A	N/A	N/A	N/A
4080	M	6 Dec 2004	N/A	N/A	N/A	N/A
4087	M	6 Dec 2004	N/A	N/A	N/A	N/A
$\bar{x}$				149†		229†

*implantation surgery

†Excluding implantation surgery dates

Table 2. Weight loss during the interval of transmitter implantation for all frogs tracked during 2004 that underwent transmitter removal.

Animal ID	**Sex**	**Mass (g) before implantation**	**Mass (g) following extraction**	**Weight change (g)**	**Percent weight loss**	**Days with implant**
0330	M	9.90	8.49	-1.41	14.24	28
0881	F	14.93	12.13	-2.80	18.75	15
0895	F	14.46	14.35	-0.11	0.76	26
1161	F	12.20	10.34	-1.86	15.25	23
1720	M	9.19	9.17	-0.02	0.22	30
4074	F	13.58	12.30	-1.28	9.43	27
4080	F	11.60	10.93	-0.67	5.78	30
4087	M	11.19	10.32	-0.87	7.77	25
$\bar{x}$		12.13	11.00	-1.13	9.02	25.50

Figure 1. Individual #0895 showing positioning for surgery, and the location/length of incision (**A.**).

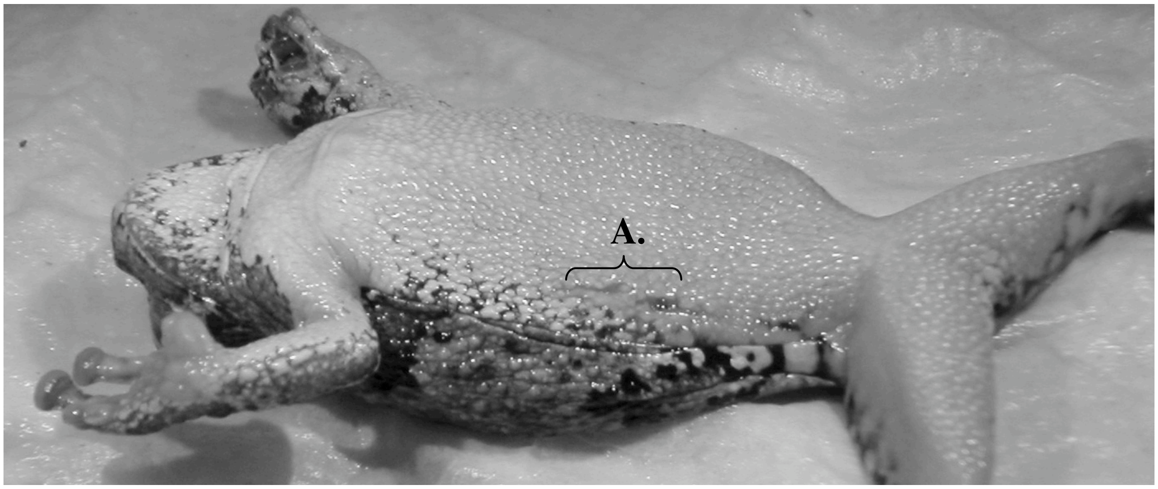
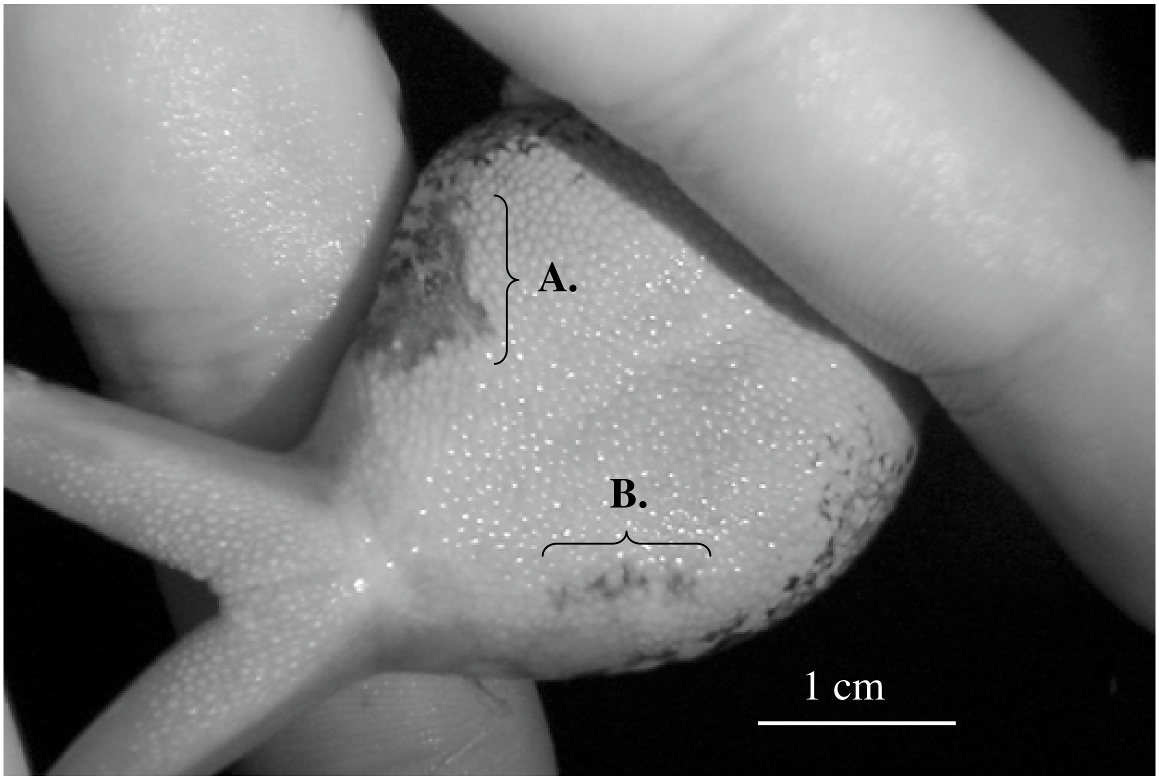

A.

Figure 2. Individual #0769 showing: **A.** scarring of early surgery with insufficient epidermal stitches 204 days post-surgery, and **B.** scarring of transmitter removal surgery with use of 9 epidermal stitches and VetBond™ 173 days post surgery.


A.
B.
1 cm

Appendix C

DIET OF THE GRAY TREEFROG (*HYLA VERSICOLOR*) IN RELATION TO FORAGING SITE CHARACTERISTICS, WITH COMMENTS ON STOMACH-FLUSHING TECHNIQUE

Rachel D. Mahan and Jarrett R. Johnson

INTRODUCTION

Currently, habitat destruction poses a significant widespread threat to amphibian population persistence (Blaustein and Kiesecker 2002). Because many amphibians have a biphasic lifestyle split among aquatic and terrestrial habitats, it is crucial to gain a better understanding of life history processes in each habitat type in order to perceive the effects of changes in the environment on amphibian populations. Most studies have focused on activities performed at breeding sites during the breeding season, because large congregations of breeding adults are relatively easy to sample. However, less is known about the habits of amphibians within the terrestrial habitat adjacent to breeding sites. The gray treefrog, *Hyla versicolor*, is found in the eastern half of the United States (Conant and Collins 1998), and has been proposed as the apex predator of treehole food webs (Park et al. 1950). However, Park et al. (1950) never encountered treefrogs in the treeholes they examined, and it is unclear as to where the individuals used in their report of treefrog diet were found. The data presented herein describe the diet of the gray treefrog as observed from individuals captured in artificial arboreal refugia (Appendix A) in the terrestrial environment and at breeding ponds.

Studies of amphibian diets vary in terms of the techniques utilized. Frogs may be euthanized and stomachs extracted (Berry and Bullock 1962; Jenssen and Klimstra 1966; Oplinger 1967; Lamb 1984; Donnelly 1991; Marshall and Camp 1995), anesthetized and contents removed with forceps (Hirai and Matsui 1999, 2000, 2001), or flushed with forcible injection of water through an inserted catheter (Legler and Sullivan 1979; Freed 1982; Leclerc and Courtois 1993). Authors who employ the stomach-flushing technique generally cite Legler and Sullivan (1979), but they provide little detail regarding the technique as it applies to anurans. Other research describing the stomach-flushing procedure for anurans focus on larger species in the genus *Rana* (e.g., Leclerc and Courtois 1993), and standard stomach-flushing techniques required some modification for use on gray treefrogs. Herein, we provide detailed information regarding the stomach-flushing technique, and data illustrating how the composition and quantity of treefrog diet samples varies with aspects of foraging site location such as distance from breeding ponds, and refuge-tree diameter.

METHODS

Study Species

Gray treefrogs are nocturnal, moderately sized frogs (32-51 mm SVL) (Conant and Collins 1998) that are extremely abundant in central Missouri. Frogs breed from April to mid-July, and males are found calling from low branches or bushes surrounding fishless, ephemeral ponds (Johnson 2000). Individuals leave breeding choruses on occasion to forage in the terrestrial habitat and replenish their energetic reserves (Gerhardt et al. 1987; Ritke and Semlitsch 1991; Murphy 1994a, b). Females return to ponds once or twice per breeding season to deposit eggs (Ritke et al. 1990; Johnson 2000). Most times of year, including the breeding season, treeholes are utilized as diurnal retreat sites (Johnson et al. Chapter 2A). Treeholes may also be utilized as sources of prey during nocturnal foraging bouts (Park et al. 1950), both during the breeding and non-breeding seasons.

Study Area

This study was conducted at three breeding locations within the Baskett Wildlife Research Area in Boone County, Missouri from 13 June until 15 July 2004. We captured treefrogs by hand during nightly visits to breeding choruses, and in artificial arboreal refugia made of ABS conduit. These pipe refugia were placed three meters high in deciduous trees (*N*=100) in transects extending up to 200 m into the terrestrial habitat (Johnson Appendix A). Artificial refugia were utilized by gray treefrogs in place of natural treeholes, and were checked during daylight hours when frogs were inactive. Terrestrial habitat adjacent to breeding sites consists of an oak/hickory canopy (*Quercus* spp./*Carya* spp.) with a maple/cedar understory (*Acer* spp./*Juniperus* spp.).

For each frog captured for stomach content analysis, we recorded snout-vent length (SVL; to nearest 0.5 mm) using a plastic ruler and body mass (BM; to nearest 0.1 g) using hand-held Pesola spring scales. Each individual was either recorded as a recapture or given a unique mark via toe excision. Each individual was then flushed (see below) twice to assure all contents (if any) were removed. Extracted stomach contents were preserved in 95% ethanol and returned to the lab. Stomach contents were dried on filter paper and weighed to the nearest 0.001 g using a digital laboratory scale.

We compared the average stomach content masses of pond captured frogs versus refugia captured frogs with two-tailed T-tests. Using simple linear regression, we analyzed the effect of distance from breeding ponds and refugia-tree diameter on stomach content mass. And we used a G-test to determine the effect of tree species on average mass of stomach contents. All stomach content mass data was natural log-transformed to meet the assumption of normality.

Stomach Flushing Techniques

The frog's forelegs were held between researcher's index and middle finger. The hind legs were immobilized with the rest of the fingers. The researcher's thumb was then pressed lightly between the eyes to provide resistance while a catheter tube (<1 mm inside diameter) was used to push up on the rostrum to force the mouth to open slightly for insertion of the catheter between premaxilla and mandibular symphysis. Once inside, the catheter was slid slowly down the esophagus and into the stomach (located to the left side of the body cavity). A small amount of water squeezed from an attached 60 cc syringe was used as lubricant. The catheter was inserted until met with slight resistance and then was drawn back slightly to avoid rupture of the stomach lining with the injection of water. Once the tube was inserted, the treefrog was oriented with its head downward. The researcher's thumb could then be used to hold open the mouth while ~60 cc of DI and UV-filtered water were flushed at a gentle but medium pace through the syringe and catheter, and into the frog's stomach.

Even if contents were produced, the stomach-flushing procedure was repeated two more times, with ~60 cc of water each, to standardize the procedure. Flushing usually produced contents in a large bolus along with small fragments of prey, which emptied into a small dish. Extracted stomach contents were removed from the water using forceps, preserved in 2.0 ml vials with 95% EtOH, and returned to the lab for analysis. We assumed that the stomach-flushing technique successfully removed all stomach contents because continued bouts of flushing subsequent to bolus removal did not produce additional items.

RESULTS

From 13 June until 15 July, we stomach-flushed 120 individuals a total of 179 times, obtaining 134 diet samples and a total of 409 individual prey items ($\bar{x}$=3.33, range=0-14). Of the 45 individuals lacking stomach contents, 37 were males at breeding ponds. Only 3% of females and 6% of males caught in refugia lacked stomach contents, compared to 66% and 74% respectively, at breeding ponds. The stomachs of 36 individuals contained plant matter (11 contained plant material only), and in seven instances stomach contents were too digested to be identified. The remaining 116 diet samples contained 2 subphyla, 4 classes, 14 orders, and 24 families (Table 1). Members of the family hymenoptera were further classified into 2 subfamilies, 3 genera, and 2 species (Table 2). Hymenopterans were the most numerous followed by Coleopterans, occurring in 99 and 94 of the stomachs, respectively. Formicidae was the most common family and the only family found in the order Hymenoptera.

Raw stomach content masses indicated that females consumed a greater prey biomass than did males ($T_{2\text{-tail}}$=2.711, *df*=73, *P*=0.008; Fig. 1A), but when standardized by length-specific mass, males and females consumed similar amounts of food ($T_{2\text{-tail}}$=2.012, *df*=46, *P*=0.452; Fig. 1B). Further, larger males consumed a greater proportion of their length-specific masses than did smaller males ($F_{1,67}$=12.230, *P*<0.001; Fig. 2), but females do not illustrate this relationship ($F_{1,31}$=0.019, *P*=0.891; Fig. 2). Individuals captured in artificial refugia contained greater masses of stomach contents than individuals captured at ponds for both males ($T_{2\text{-tail}}$=1.997, *df*=64, *P*=0.003; Fig. 3) and females ($T_{2\text{-tail}}$=2.145, *df*=14, *P*=0.006; Fig. 3). The distance of refugia from breeding ponds positively affected the masses of stomach contents for females ($F_{1,26}$=5.438, *P*=0.028; Fig. 4), but not males ($F_{1,28}$=1.439, *P*=0.240; Fig. 4). Furthermore, refugia-tree diameter positively affects the stomach content masses of males ($F_{1,28}$=3.748, *P*=0.063; Fig. 5) but not females ($F_{1,26}$=0.442, *P*=0.512; Fig. 5). There is an apparent, but not significantly significant effect of tree species on average stomach content mass (G=2.190, *df*=4, *P*=0.701; Fig. 6).

DISCUSSION

Our study revealed that *H. versicolor* consumes a wide variety of terrestrial animals, mostly arthropods. No species limited to aquatic habitats was extracted, indicating that *H. versicolor* forages exclusively in the terrestrial environment. The high abundance of adult insects and absence of recently metamorphosed insect nymphs provides further evidence of the importance of terrestrial habitat surrounding wetlands. Although, as reported by Horn and Ulyshen (2004) for *H. cinerea*, we observed Coenagrionid damselflies in stomach contents, suggesting some foraging may occur very close to aquatic habitat.

Of the stomachs with contents, 31.6% contained Hymenopterans (i.e., ants) and 30.0% contained Coleopterans (i.e., beetles). Because the beetles identified are much larger than the ants observed, beetles represent a greater proportion of the invertebrate biomass ingested by treefrogs, and thus may be the most important component of their diet. These relatively high proportions either demonstrate greater preference for, or greater availability of these prey items, though no studies of prey availability have been conducted. Plant matter, including cedar (*Juniperus* spp.) needles and seeds, was found in 20.1% of the stomachs. It is most likely that plant matter was ingested inadvertently when animal prey was captured. Although most adult frogs are carnivorous (Duellman and Trueb 1986), da Silva et al. (1989) report frugivory and seed dispersal in a neo-tropical treefrog.

We found that although female stomach contents weighed more than male stomach contents, prey consumption did not differ among males and females relative to their body sizes. This is surprising considering the potential increased energy demands required of females producing egg clutches compared to the energy required for calling by males. Although we cannot be certain of the reproductive status of individuals because many samples were extracted after the peak of breeding activity had passed. It is similarly unclear why larger males consumed food representing a greater proportion of their body size than smaller males, but this relationship could be related to size-related variation in calling effort.

Individuals captured in refugia contained more stomach contents than those captured at breeding sites. This supports the contention that treefrogs leave breeding choruses to forage at locations distant to breeding sites, and foraging activity is minimal within the confines of breeding ponds. Our data also showed that stomach content masses shared a positive association with distances from breeding ponds for females but not for males, and indicates that higher quality foraging grounds exist at greater distances from breeding sites. Johnson et al. (Chapter 2A) have found that females travel further than males, and these data support their contention that higher quality foraging sites are acquired by females that migrate further into terrestrial habitat. It is unknown, however, whether this relationship is the result of variation in prey abundances, or reduction in conspecific competitor density. The lack of an effect of distance on male stomach content masses may be the result of their relative proximity to breeding sites while foraging (Chapter 2A), and reliance on poorer quality foraging grounds.

The characteristics of trees in which refugia were located also affected average stomach content masses, however, the effects of refugia-tree diameter differed among males and females. We found that males foraging in larger diameter trees contained greater masses of stomach contents, but we elucidated no effect of diameter on female stomach content masses. Johnson and Mahan (Chapter 2B) found biases for males to use red oaks and females to use white oaks more frequently than expected at random, and that both species used larger diameter trees more frequently than small diameter trees. Because females seem to prefer white oaks, but female stomach content masses were independent of diameter, white oaks may be better foraging sites than red oaks over a wide range of tree sizes. Further, Johnson et al. (Chapter 2A) suggest that gray treefrog populations are evenly distributed throughout habitat adjacent to breeding sites, and Johnson (Appendix A) proposes that territoriality at refugia could be contributing to spacing individuals in terrestrial habitats. If territorial interactions are driven by body size, males may be relegated to inferior foraging sites (i.e., red oaks) and illustrate the effect of tree diameter on foraging site quality. Our data regarding average stomach content mass for each category of tree revealed an apparent trend for greater masses in white oaks than all other tree types, but this relationship was not statistically significant. Experimental manipulation of foraging site characteristics would help to further elucidate the relationships among habitat variables and foraging success of treefrogs.

Like most frogs, gray treefrogs appear to be generalist predators, with a diet superficially similar to that of other arboreal anurans (e.g., Johnson and Bury 1965; Kilby 1945; Oplinger 1967; Hirai and Matsui 1999, 2000, 2001). Although arboreal pipe refugia were designed to simulate natural treehole conditions, it is unclear if prey availability in and around artificial refugia is comparable with that of natural refugia. Not only should future studies investigate seasonal differences in diet and prey availability, but also, the differences in availability between natural and artificial refugia need to be taken into account. Knowledge of amphibian diets in both aquatic and terrestrial habitat during each life history stage is an important aspect of quantifying the impact that anthropomorphic habitat changes such as selective logging and insecticide pollution will have on the local population dynamics and long-term persistence of amphibians.

ACKNOWLEDGEMENTS

We thank J. Carrel for gut flushing supplies, M. Jackson for invertebrate identification, and J. Millspaugh for access to field sites. We thank R. Semlitsch and J. Carrel for thoughtful comments on early versions of this manuscript. Funding provided by TWA scholarships and a GAANN Fellowship to JRJ. RDM was supported by LSUROP. All procedures were approved by the University of Missouri Animal Care and Use Committee (protocol #3950).

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Table 1. Classification of prey items. Number of individuals indicates the number of stomachs containing prey. Stomachs with multiple species are counted more than once.

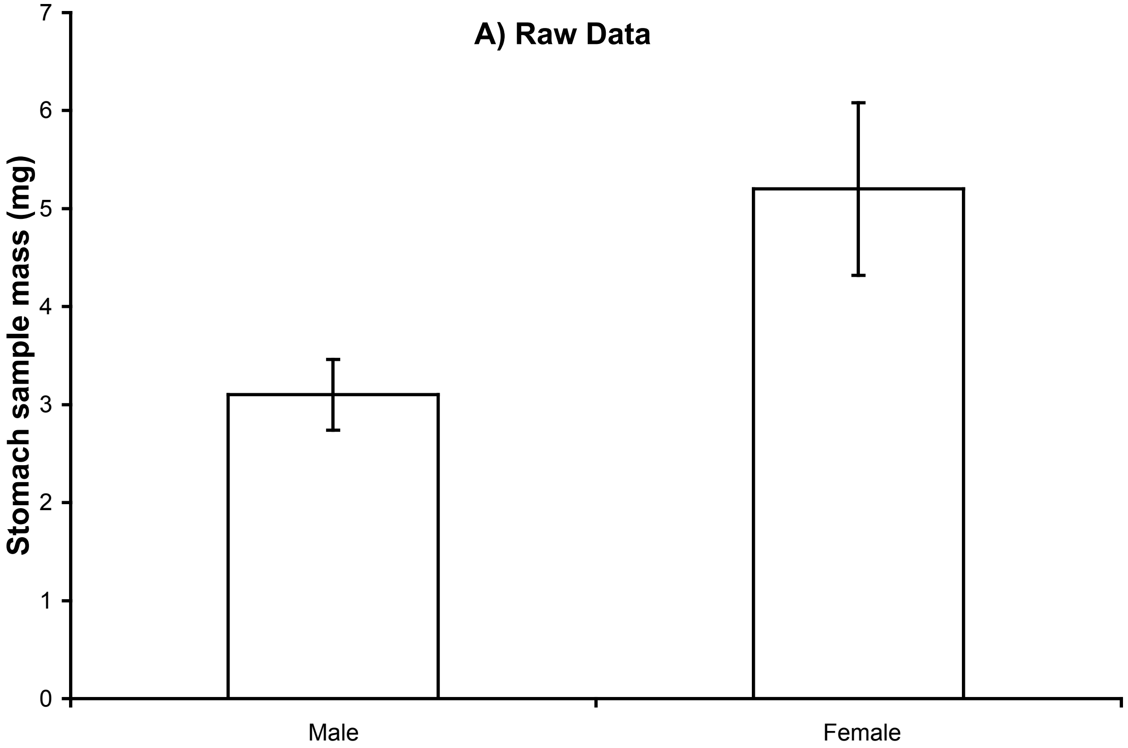
Classification	**# of items**	**% of total items**	**# of individuals**	**% of total stomachs**
Mandibulata				
Insecta				
Hymenoptera				
Formicidae	146	35.70	99	31.63
Diptera				
Unidentified	3	0.73	3	0.96
Larvae	1	0.24	1	0.32
Lepidoptera				
Noctuidae	14	3.42	11	3.51
Larvae	25	6.11	21	6.71
Coleoptera				
Unidentified	34	8.31	27	8.63
Staphylinidae	1	0.24	1	0.32
Scarabaeidae	19	4.65	11	3.51
Elateridae	22	5.38	19	6.07
Lycidae	1	0.24	1	0.32
Buprestidae	1	0.24	1	0.32
Bostrichidae	1	0.24	1	0.32
Cerambycidae	1	0.24	1	0.32
Bruchidae	2	0.49	2	0.64
Cleridae	3	0.73	3	0.96
Cucujidae	1	0.24	1	0.32
Coccinellidae	1	0.24	1	0.32
Curculionidae	20	4.89	12	3.83
Carabidae	11	2.69	9	2.88
Larvae	4	0.98	4	1.28

Classification cont…	# of items	% of total items	# of individuals	% of total stomachs
Neuroptera				
Raphididae	1	0.24	1	0.32
Hemiptera				
Unidentified	1	0.24	1	0.32
Cicadellidae	1	0.24	1	0.32
Pentatomidae	7	1.71	4	1.28
Orthoptera				
Tettigonidae	1	0.24	1	0.32
Gryllidae	3	0.73	3	0.96
Acrididae	5	1.22	5	1.60
Phasmida				
Phasmatidae	1	0.24	1	0.32
Dictyoptera				
Blatellidae	28	6.85	21	6.71
Odonata				
Coenagrionidae	4	0.98	4	1.28
Crustacea				
Isopoda	2	0.49	2	0.64
Diplopoda	1	0.24	1	0.32
Chelicerata				
Arachnida				
Araneida	9	2.20	7	2.24
Acarina	1	0.24	1	0.32
Phalangida	17	4.16	17	5.43
Unidentified	16	3.91	14	4.47
Total	409	100.00	313	100.00

Table 2. Further classification of the order hymenoptera. Percentages reflect total of all items or stomachs, not just those containing hymenopterans.

Classification	**# of items**	**% of total items**	**# of stomachs**	**% of total stomachs**
Hymenoptera				
Formicidae				
Unidentified	46	11.25	30	9.58
Formicinae				
Camponotus				
pennsylvanicus	25	6.11	19	6.07
americanus	25	6.11	18	5.75
Unidentified	19	4.65	11	3.51
Myrmicinae				
Crematogaster	14	3.42	5	1.60
Aphaenogaster	8	1.96	4	1.28
Unidentified	8	1.96	11	3.51
Larvae	1	0.24	1	0.32
Total	146	35.70	99	31.63

Figure 1. **A)** Comparison of male and female average stomach content masses (±SE), and **B)** average stomach content masses standardized by length specific mass (mg/mm; ±SE).


A) Raw Data
Stomach sample mass (mg)
0
1
2
3
4
5
6
7
Male
Female

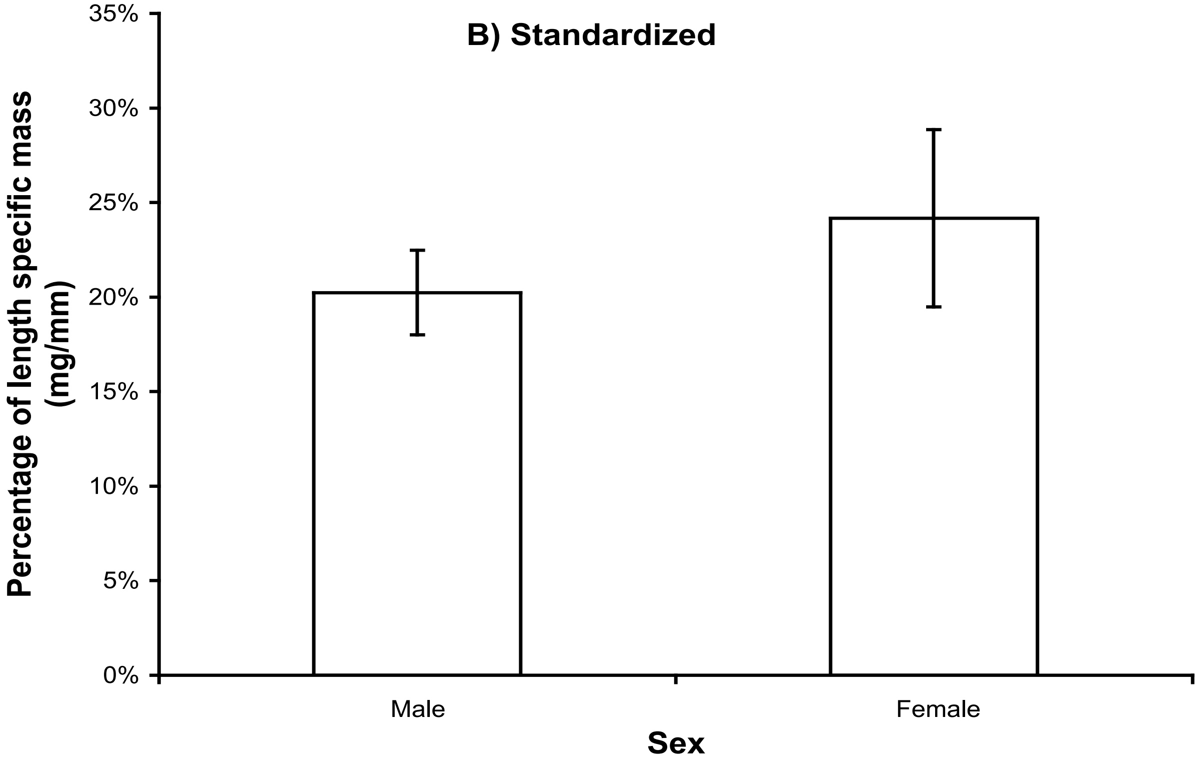
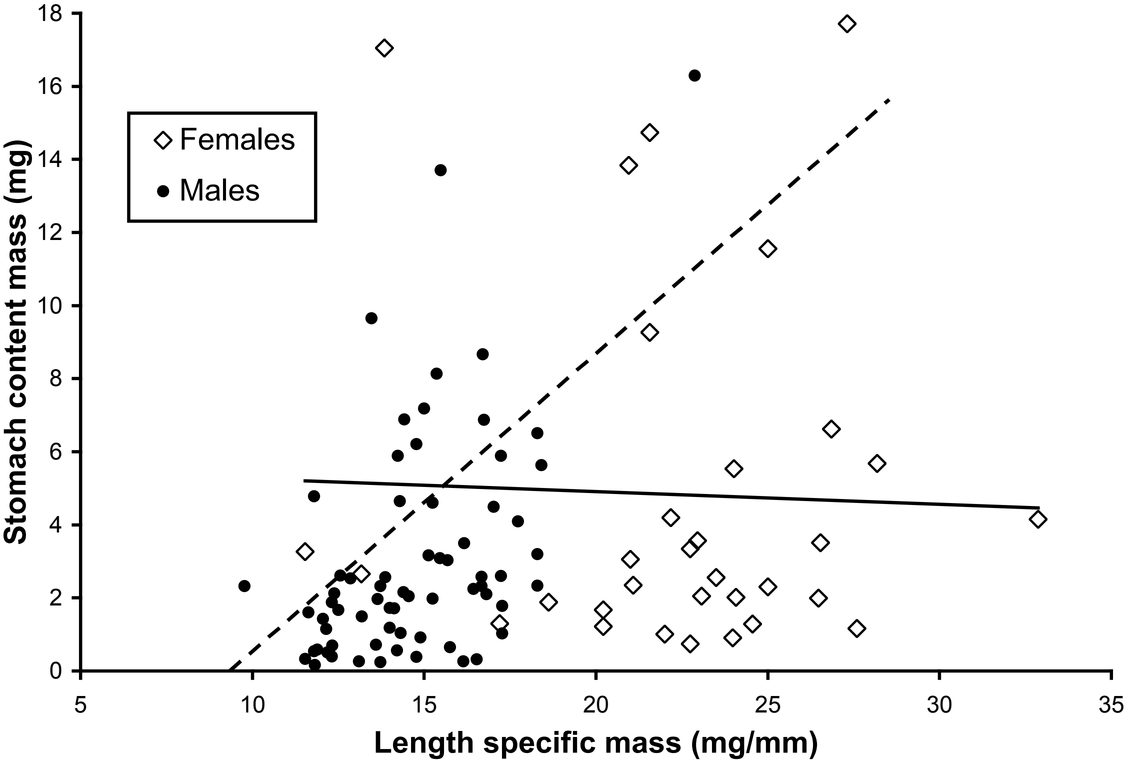

B) Standardized
Percentage of length specific mass (mg/mm)
0%
5%
10%
15%
20%
25%
30%
35%
Male
Female
Sex

Figure 2. Effect of length-specific mass (mg/mm) on stomach content masses of females (solid line) and males (dashed line).



Stomach content mass (mg)
0
2
4
6
8
10
12
14
16
18
◇ Females
● Males
Length specific mass (mg/mm)
5
10
15
20
25
30
35

Figure 3. Comparison of average stomach content masses for pond and refugia captured males and females (±SE).

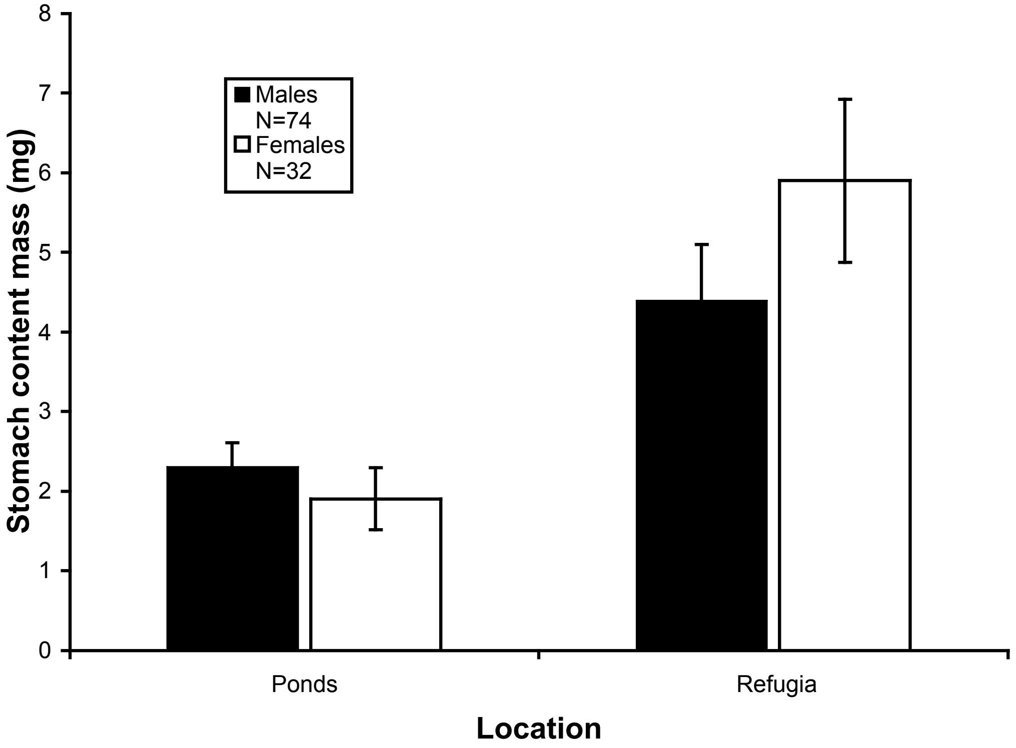

8
7
6
5
4
3
2
1
0
Stomach content mass (mg)
■ Males
N=74
□ Females
N=32
Ponds
Refugia
Location

Figure 4. Scatter-plot of effect of refugia distance on male (dashed line) and female (solid line) stomach content masses.

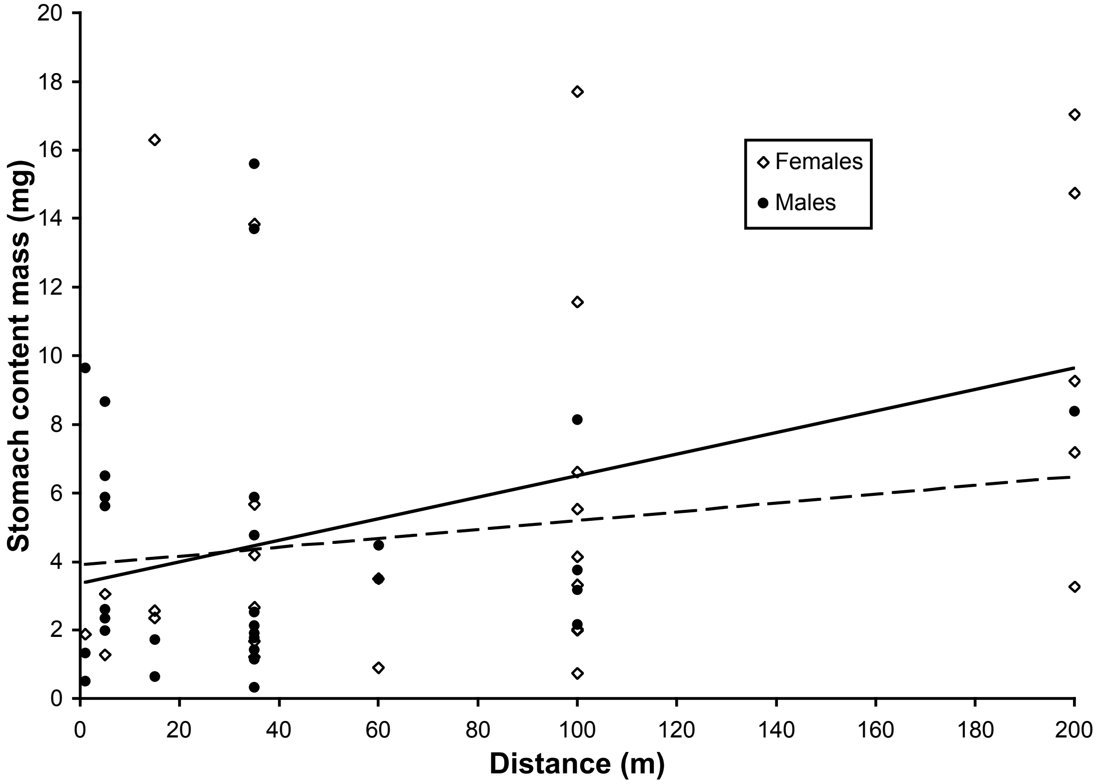
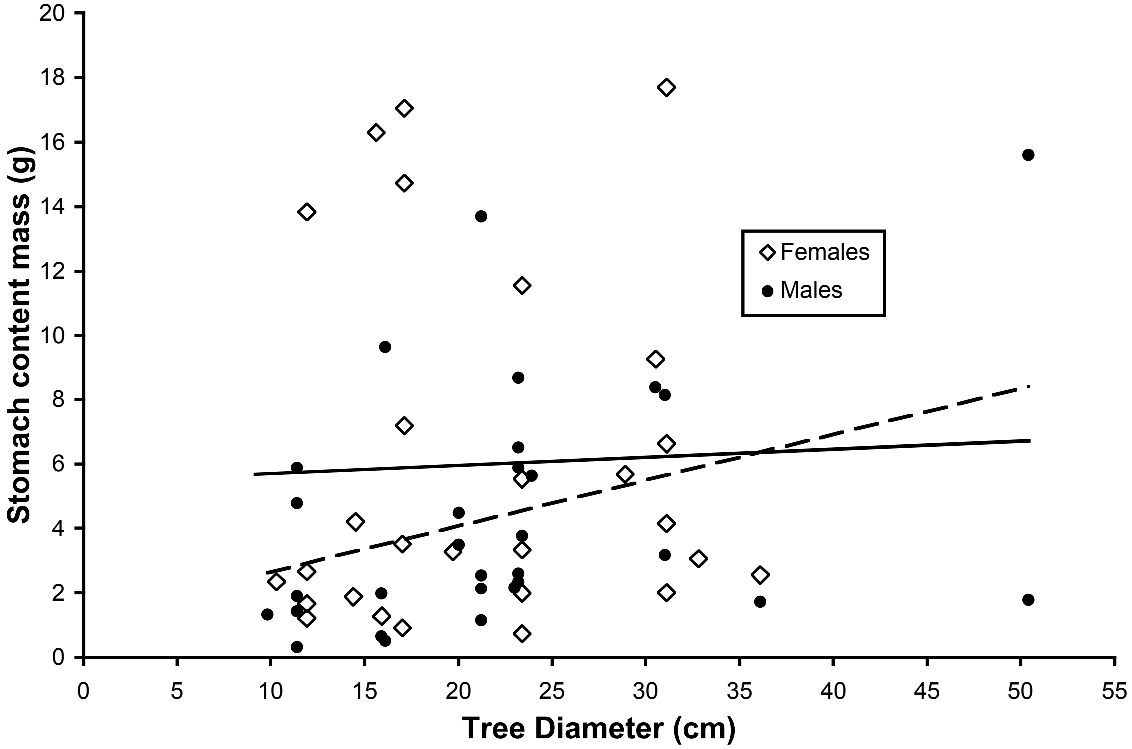
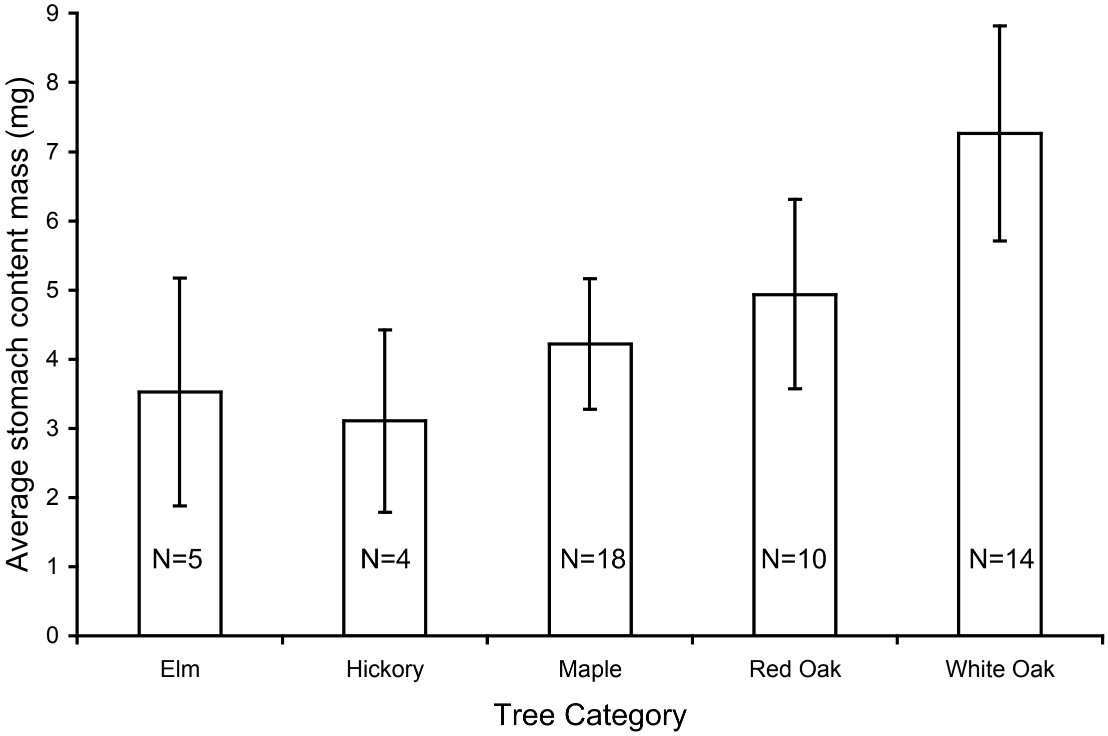

Stomach content mass (mg)
0
2
4
6
8
10
12
14
16
18
20
Distance (m)
0
20
40
60
80
100
120
140
160
180
200
◇ Females
● Males

Figure 5. Scatter-plot of effect of tree diameter on male (dashed line) and female (solid line) stomach content masses.



Stomach content mass (g)
0
2
4
6
8
10
12
14
16
18
20
Tree Diameter (cm)
0
5
10
15
20
25
30
35
40
45
50
55
◇ Females
● Males

Figure 6. Average stomach content masses by each tree species category (±SE).


Average stomach content mass (mg)
9
8
7
6
5
4
3
2
1
0
N=5
N=4
N=18
N=10
N=14
Elm
Hickory
Maple
Red Oak
White Oak
Tree Category

VITA

Jarrett Reed Johnson was born on 17 May 1977 in the west Chicago suburb of Winfield and spent the majority of his childhood on the Illinois side of the Wisconsin border in the small town of Wadsworth. Following his graduation from Warren Township High School in Gurnee, Illinois, in 1995, he enrolled at the University of Illinois' Champaign-Urbana campus. Jarrett earned a B.S. from the Department of Ecology, Ethology, and Evolution with honors and distinction in May 1999, and was awarded the outstanding undergraduate achievement award. He then enrolled at the University of Missouri's Columbia campus in August 1999. In September 2003 he married Gina M. Woloszyn and earned his Ph.D. from the Division of Biological Sciences in July 2005 with an emphasis in Ecology and Evolution. Jarrett's future plans include conducting post-doctoral research in the fields of evolutionary biology or population ecology, with the ultimate goal of obtaining a research-oriented faculty position.